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HÆMORRHAGIC SEPTICÆMIA IN MAN DUE TO CAPSULATED BACILLI.*

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In this paper it is my purpose to consider only those cases of septicæmia accompanied by hæmorrhages into the skin, the serous membranes, and usually into the liver, spleen, kidney, heart, stomach and intestines in which capsulated bacilli have been found as the only, or, at least, the most numerous and important micro-organisms present.

These restrictions remove from our consideration cases of primary septicæmia with hæmorrhages, as well as the numerous instances of secondary hæmorrhagic septicæmia occurring in variola, typhoid fever, scarlatina, rheumatism, measles, tuberculosis, endocarditis, nephritis, puerperal fever, omphalitis, and the like, due for the most part to streptococci, staphylococci, or various bacilli, examples of which have been reported by Klebs (16), Hlava (14), Babès (2), Martin de Gimard (19), Claisse (9), Legendre and Claisse (18), Tavel and de Quervain (21), Kamen (15), Neumann (20) and others. The so-called purpura anthracosa and purpura scorbutica are also, of course, excluded.

It is difficult to say by whom the class of cases with which we have to do was first described. Although some of the early examples reported

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by Ceci (7) in 1881, and by Klebs (16) in 1887, and that of Watson Cheyne (8) in 1883, in which bacilli were found, probably come within the scope of this article, the descriptions of the microscopical lesions and of the bacteria found are too scanty to warrant their consideration here.

In 1887 Bordoni-Uffreduzzi (6) described three cases of a peculiar septicæmia, fatal in from two to four days, with hæmorrhage into and congestion of the internal organs. In each instance he found the same micro-organism, a pleomorphic capsulated bacillus pathogenic for dogs, mice, rabbits and guinea-pigs. He called it *Proteus hominis capsulatus.* It differs in many ways from the Proteus group of Hauser. This organism was about the size of the anthrax bacillus, capsulated, and stained by Gram. It was found in numbers in the larger blood-vessels and among the tissue elements, but not in the capillaries.

It is thought best to exclude from our list the case described by Foà and Bonome (11), who report finding a bacillus identical with Proteus vulgaris (Hauser) in the blood and in the organs of a man dead of ileus with extensive hæmorrhagic infiltration of the small intestine and the corresponding mesentery and with thrombosis of the mesenteric vein. It is not at all clear that the "hæmorrhagic infiltration" was other than hæmorrhagic infarction of the intestine.

Banti (5) in a case of acute hæmorrhagic infection found in pure culture a bacillus which he calls *Proteus capsulatus septicus,* and which he thinks may be identical with Proteus hominis capsulatus of Bordoni-Uffreduzzi. Banti's bacillus appears to be more like the bacillus of Friedländer than was the organism of Bordoni-Uffreduzzi. The latter is regarded by Foà as Proteus vulgaris (Hauser) and not as a new bacillus.

Babès (1) in 1889 described three instances of death in children from hæmorrhagic septicæmia, with stomatitis, angina, bronchitis, anæmia and fever, in the organs of whom he found a short, non-motile, capsulated bacillus, which stained poorly with the aniline dyes and by Gram, did not form spores, was facultatively anaërobic, and grew feebly on gelatine, which was not liquefied. On agar there were small transparent drops which later turned to a whitish yellow color, and were without sharply defined margins. On potato, whitish drop-like colonies formed. Bouillon was made cloudy. Mice inoculated with this bacillus died with hæmorrhagic septicæmia; rabbits, killed by it, showed hæmorrhage into all the organs. Sterilized cultures caused multiple hæmorrhages when injected into animals. For guinea-pigs and dogs it was but feebly pathogenic.

Tizzoni and Giovannini (22) in 1889 reported three cases of hæmor-

rhagic infection in which they found a capsulated bacillus. They were in children with impetigo contagiosa and two of them were rapidly fatal. One child came to autopsy and showed œdema and hæmorrhages into the skin, hæmorrhages in the serous membranes, lymphatic glands and various internal organs. In the kidneys and liver there were areas of marked tissue degeneration and leucocytic infiltration. The bacillus found was pleomorphic, capsulated, often with rounded ends, stained by Gram and was pathogenic for dogs, rabbits and guinea-pigs, but not for white mice or for pigeons, even in large doses. In the cutaneous lesions and in some of the organs Staphylococcus pyogenes aureus was also present.

In 1891 Kolb (17) reported five cases of idiopathic purpura hæmorrhagica, three of which died in from two to four days. Cultures made from blood obtained from a vein during life remained sterile. Cultures from the various organs of the fatal cases gave always the same bacillus in pure culture. This bacillus was demonstrated in sections stained by the Kühne and the Gram-Weigert methods, being especially abundant in the spleen; it was sometimes found in the blood and lymph vessels, sometimes in the interstitial tissue of the hæmorrhagic areas and in the lymph spaces. It had usually rounded ends and was surrounded by a thin capsule, especially in the animal body. It was partly decolorized with Gram's method. The bacillus was very pathogenic for field mice, for white mice and for rabbits; less so for dogs. Guinea-pigs were affected only by large doses, while pigeons showed only local necrosis at the seat of inoculation. Hæmorrhages were found in the animals after inoculation with dead and filtered, as well as with living, cultures.

In 1891 Babès and Oprescu (4) described a fatal case of hæmorrhagic septicæmia simulating typhus fever. From the organs and urine they obtained a capsulated bacillus in pure culture. The patient, a male, aged 22, had had marked hæmorrhages into the skin. At the autopsy the tissues of the neck and of the mediastinum were œdematous, and there were hæmorrhages into the skin and serous membranes. Tissue degenerations were found in the liver, spleen and kidneys.

The bacilli, which were found in all the organs, varied much in size; they were often in pairs, were surrounded by capsules and stained poorly by Gram. The organism was pathogenic for rabbits, guinea-pigs, mice and pigeons, the animals dying with hæmorrhagic septicæmia.

In 1893 Babès (4A) reported several cases of hæmorrhagic infection, associated especially with hæmorrhagic bronchitis, and referred by him to various micro-organisms, both cocci and bacilli.

Von Dungern (10) in 1893 reported a fatal case of hæmorrhagic sep-

ticæmia in a child, two weeks old, in whose organs was found a capsulated bacillus. There was no history of syphilis or hæmophilia. Thirteen days after birth the child was taken ill with hæmorrhage from the right ear, nose and mouth. Later there appeared petechiæ over the whole body. The autopsy showed hæmorrhages in various organs, in the subcutaneous tissue of the thighs, in the abdominal cavity, and in the umbilicus. Cultures from the thrombosed umbilical artery showed a short, plump bacillus, 1 to 2 μ long and half as broad, surrounded by a capsule. This bacillus was very pathogenic for white mice, guinea-pigs and rabbits, causing a fatal hæmorrhagic septicæmia with a fibrino-purulent exudation into the serous cavities.

To the foregoing cases I wish to add two others which came under my observation at St. Joseph's Hospital, Baltimore.

In June, 1892, five persons who had been working as berry-pickers, near Annapolis, Maryland, were taken ill and came to Baltimore for treatment. One died several days later under the care of a physician who gave a death certificate for "malaria." Two others came to St. Joseph's Hospital; the remaining two could not be traced. The two patients admitted to my service at the hospital were suspected of having typhus fever. One died a few hours after admission; the other died at the Quarantine Station some ten days later.

Case I. John Y., married, aged 51, a Pole, was admitted to St. Joseph's Hospital at 3 p. m., June 13, 1892, complaining of fever and weakness.

Family history.—Father and mother dead, cause unknown. One brother and sister living, none dead. One son and three daughters living and well. Knows nothing of his grandparents. His wife was taken with the same symptoms the day before he was affected.

Personal history.—Healthy as a child. Eleven years ago had typhoid fever. No other illness.

Present illness.—Seven days ago, while picking strawberries in the hot sun (the weather was excessively hot at that time) his head became very hot and painful, and shortly afterward he was seized with pain in the epigastrium. These symptoms came on suddenly, the patient having felt perfectly well before their appearance. No history of a chill was obtained, but the patient felt hot and feverish from this time on. On the next day, June 8, his nose bled four times. No further history could be got from the patient, except that his wife and three of his fellow-workers were similarly affected.

On entrance into the hospital, the axillary temperature was 102.4° F.; pulse 90, regular, of fair volume, and not dicrotic; respirations 30. He was seen by me two hours after his entrance, when the following note was made: Large, well-built, muscular man; skin dusky, warm and dry; face heavy, apathetic; he answers questions slowly but intelligently. Pupils of normal size and react to light; conjunctivæ slightly suffused. Tongue covered on the dorsum with thin white fur, the margins bright red, the papillæ swollen. No œdema. Surface of the skin covered with a purplish macular eruption, which on the anterior surface of the chest resembles very light-colored freckles. On the back they are very numerous and red in color. On both arms, the buttocks, thighs and legs, always best marked on the extensor surfaces, is a diffusely spread, dark red, slightly papular eruption, which fades on pressure and slowly returns on its removal. It is somewhat confluent in places, but is never crescentically arranged. The individual papillæ have no definite shape. On the legs, over the tibiæ and ankles, are numerous purplish petechiæ, resembling spots of purpura hæmorrhagica. Examination of lungs negative. Cardiac dulness slightly increased; heart sounds normal. Examination of liver negative. Splenic dulness increased; spleen not palpable. No rose spots on abdomen. No gurgling or tenderness in right iliac fossa.

June 14, 10 a. m. Temperature 104.5° F. Pulse continues strong. Patient restless and at times delirious during the day. Temperature reached its highest point, 105.4° F., at 8 p. m. Urine dark red, acid, sp. gr. 1024. No albumin, sugar or casts. Moderate increase of polynuclear leucocytes in blood. No malarial parasites found. At 9 p. m. patient was taken to the Quarantine Station, and passed from my observation.

The quarantine officials informed me that the fever and cutaneous hæmorrhages persisted, and that death occurred ten days after his removal from the hospital. The report of the coroner's physician, Dr. Keirle, who made the autopsy, states that there were hæmorrhages into the skin and serous membranes, with marked congestion of the lungs, liver, spleen, heart, kidneys, brain and cord, and acute parenchymatous degeneration of the heart, liver and kidneys. No cultures were made.

Case II. Julia Y., a Russian Pole, 45 years old, was admitted to St. Joseph's Hospital with her husband (Case I) June 13, 1892, complaining of fever and epigastric pain. Family history negative; personal history good.

On June 7, while picking strawberries in the hot sun, she was suddenly seized with great pain in the head, epigastrium and chest. She

felt very hot all over, but especially in the head. The same day she broke out with a slightly papular eruption which was spread diffusely over the body. No history of a chill was obtained. She was seen by me two hours after her entrance, when the following note was made: Well-built woman, of medium height. Conjunctivæ of both eyes, especially of the lower lids, injected. Both corneæ slightly clouded, pupils moderately dilated. Skin of the nose and face somewhat swollen, but not œdematous. Tongue swollen and painful to the touch, its dorsum covered with yellowish white fur, the papillæ prominent, edges red. Small whitish ulcers on the inner side of each lip. Patient lies on the back, is dull and apathetic, answers questions with difficulty. Temperature on admission 101.4° F.; pulse 126, slightly irregular, weak, low tension, not dicrotic. Respirations 45, shallow, costo-abdominal. No enlargement of lymphatic glands. Skin warm, moderately dry; its surface covered everywhere with thick and diffusely spread papular eruptions. In some places, as on the anterior surface of the chest, the papules are small, dark red, irregularly shaped, about the size of a pin's head, not fading on pressure. In some places there are true petechiæ; in others, particularly on abdomen, surface covered with macular eruption, appearing as a fine dark mottling distributed in irregular streaks. Papular eruption predominates over back, anterior surface of thighs and buttocks. On arms and forearms, best marked over deltoid regions and posterior surface of elbow joint, is a diffuse, red, thickly scattered, small papular eruption, very irregular in shape, and in places somewhat concentrically arranged, very slightly modified by pressure. Skin of thighs, legs, and anterior surfaces of ankles and feet presents a widespread petechial eruption. On chest numerous small sudaminal vesicles. Skin over lower extremities covered with sweat. Examination of heart and lungs negative. Liver dulness begins at lower border of 6th rib in mammary line, and extends 3 cm. below costal margin. Edge of liver palpable, round and smooth. Splenic dulness noticeably increased; edge of spleen not easily palpable below costal margin. Much tenderness over abdomen, which is everywhere resonant; no special tenderness or gurgling in right iliac fossa.

June 14, 10 a. m. Patient decidedly worse this morning; pulse 108, weak, of poor volume, irregular and dicrotic; temperature 100.4° F., respirations 30. Patient was restless and delirious throughout night, and was kept in bed with difficulty. Bowels constipated. Urine scanty; specimen drawn with catheter is pale yellow, acid, specific gravity 1023, free from albumin, sugar, and casts. No characteristic diazo-reaction; no hæmaturia or hæmoglobinuria. Death occurred at 4.15 p. m., pre-

ceded by great rise of temperature (109.6° F. at 4 p. m.), rapid, weak, dicrotic pulse and persistence of the eruption.

Autopsy (5 hours after death). Anatomical diagnosis: Hæmorrhagic septicæmia; acute splenic tumor; fluid blood in the heart and all the blood-vessels. Acute congestion of the lungs, liver, spleen, kidneys, pancreas, stomach and intestine. Cloudy swelling of liver and kidneys. Fatty degeneration of heart, liver and kidneys.

Rigor mortis present. On skin of abdomen there is a fine macular eruption of a mulberry hue. Here and there are scattered petechiæ and several "taches bleuâtres." On posterior and inner surfaces of arms and lower extremities and on back purplish-blue post-mortem discoloration. On anterior surface of upper and lower extremities numerous large and small petechiæ, which do not disappear on pressure. Superficial lymphatic glands not swollen. Muscles dark red.

Brain.—Dark fluid blood, without clots, in sinuses. Hyperæmia and œdema of brain and meninges. *Abdomen:* Moderate hyperæmia of peritoneum. A few scattered subperitoneal ecchymoses. Intestinal surface dark red, mesenteric glands swollen and dark red. *Thorax:* Marked hyperæmia and numerous small subpleural ecchymoses, especially over lower lobes. Lungs very hyperæmic and œdematous. Muco-pus in bronchi. Clear serum in pericardial cavity. Small ecchymoses beneath epicardium. Dark fluid blood in all cardiac cavities. Myocardium flabby and pale. Valves normal. Arteries normal, contain dark, fluid blood. *Liver:* Much swollen, very hyperæmic, of mottled dark red and opaque grayish color, and soft consistence, without visible focal necroses. It presents small scattered hæmorrhages. Gall bladder contains thick, dark greenish bile. *Spleen:* Swollen, very soft, almost diffluent, and dark red. Trabeculæ and Malpighian bodies invisible. *Kidneys:* Very much swollen, especially the cortex, of a dark red color, and softened in consistence. Cortical striæ obscure, opaque; Malpighian tufts dark red and prominent; pyramids very hyperæmic. Capsule not adherent. *Pancreas* and *adrenals:* Show no especial change, except congestion. *Stomach* and *intestine:* Deeply injected along their mesenteric border. Gastric and intestinal mucosæ bright red, deeply congested. Peyer's patches in upper portion of ileum swollen and, with the solitary follicles, red. Peyer's patches just above the ileo-cæcal valve somewhat swollen and present a shaven-beard appearance. No ulceration. *Uterus* of normal size, with soft, red and congested mucosa. Ovaries and Fallopian tubes normal. *Bladder:* Contains blood-stained urine and its mucosa is red and injected. *Thyroid:* Twice the normal

size, being symmetrically swollen, is dark red and very much congested. Its consistence is soft.

Microscopical Examination.—Fresh frozen sections and sections of tissues hardened in Flemming's solution, in alcohol, and in Müller's fluid were studied. Sections of hardened tissues were stained by safranin, hæmatoxylin and eosin, lithium carmin, Weigert's fibrin stain, and methylene-blue and eosin.

Liver.—There was widespread fatty degeneration of the hepatic cells, as well as of the endothelial cells of the intralobular capillaries and of some of the large veins. The liver cells were swollen and granular; many contained much yellow pigment. The capillaries were everywhere dilated, being filled with red corpuscles, among which were many large mononuclear and polymorphonuclear leucocytes. The central veins of the lobules and the neighboring capillaries were widely dilated, the liver cells about them being flattened by pressure. The capillary endothelium was swollen. All the branches of the portal and hepatic veins were dilated. In some of the larger veins were seen many long cells with elongated nuclei. The hepatic veins contained liver cells, singly and in clumps of two, three or even more. There were a few small intralobular areas of infiltration with lymphoid cells, some short fusiform cells, and some polymorphonuclear leucocytes. There was some increase of the interlobular connective tissue. Larger and smaller areas of hæmorrhage were present within the lobules. In some places only a few red corpuscles were seen among the liver cells, while in others the hæmorrhages occupied as much as the third of a lobule. Both in the veins and in the capillaries were to be seen short and long bacilli, occurring both singly and in small groups. Here and there they occurred also free in the tissue, being numerous in the areas of hæmorrhage and of round-cell infiltration. No capsules were definitely detected, and the bacilli were never observed within cells.

The *heart* showed fatty degeneration of the muscular fibres, with great dilatation of the capillaries and small veins, in both of which bacilli could be seen.

Sections made from several portions of each *lung* showed engorgement of all the blood-vessels with blood. In the lower lobes there was marked œdema, with great dilatation of the capillaries and veins. There was no inflammatory exudation in the air vesicles. In some of the small arteries and dilated capillaries liver cells were seen. Bacilli were found in the blood-vessels but not in the tissues.

The vessels of the *spleen* were enormously dilated and there were numerous large and small hæmorrhages in the splenic pulp. Within

the blood-vessels and in the hæmorrhagic areas bacilli in large and small clumps or masses were seen. They never completely plugged the capillaries. In the veins and occasionally in the capillaries liver cells singly and in short rows were found; these nowhere, however, seemed to fill the lumina of the vessels. Lying free in the tissue spaces and often inside of cells, both polymorphonuclear leucocytes and lymphocytes, were larger and smaller yellow pigment masses, which appeared not only as fine granules, but also in large irregular masses. This pigment stained deeply with eosin and with picric acid. There was no special hyperplasia of the splenic tissue, but the polymorphonuclear leucocytes were increased in number, both in the blood-vessels and in the tissues. There were definite areas of tissue necrosis. The red corpuscles both in the vessels and in the areas of hæmorrhage were pale and stained poorly.

Kidneys.—Fresh frozen sections of the kidneys showed cloudy swelling and fatty degeneration of the epithelial cells of the convoluted tubules and of the glomerular epithelium. In sections hardened in Flemming's solution fat drops were noted in the endothelial cells of the glomerular capillaries. The blood-vessels, both large and small, in all parts of the organs were greatly distended with blood. There was a notable increase of the large mononuclear as well as of the polymorphonuclear leucocytes. In many of the veins liver cells were present without occluding completely the lumen. The glomerular capillaries were widely dilated and contained an increased number of cells. The glomeruli were swollen and completely filled their capsules. The epithelial cells of the convoluted tubules were swollen, very granular and often devoid of nuclei; some were desquamated. There was no hæmorrhage into the glomerular capsules or into the tubules. There were a few small areas of round-celled infiltration with atrophy of the renal tissue, and a slight amount of arterio-sclerosis of the smaller vessels. Except in the large blood-vessels, few bacilli were to be found in the sections of the kidney. None were seen free in the tissues.

In the *stomach* and *intestine* there was marked dilatation of the vessels, especially of the serous, mucous and submucous coats. In the ileum there was hyperplasia of Peyer's patches and of the diffusely spread lymphoid tissue. The mesenteric lymphatic glands showed congestion and histological changes similar to those in the spleen.

The *thyroid gland* showed marked hypertrophy, the veins and capillaries being very much dilated. Bacilli were found in the vessels.

Bacteriological examination.—The bacterial forms found were the same in all the organs, and were most numerous in the blood-vessels, especially the veins. Only in the liver were bacilli found outside the

vessels. They were demonstrated best in sections stained first with lithium carmin and then with Weigert's fibrin stain. They varied much in size and in shape. Rod-shaped forms were the most common but occasionally forms suggesting large cocci and diplococci were found. Many short and long, oval forms were seen. Some of the rods were short and stout with rounded ends; others longer (1 to 2 μ), while some were long filaments (3 to 5 μ). Sometimes two or more rods were seen end-to-end, while others did not stain throughout but presented a beaded appearance. Capsulated forms were occasionally seen.

At the time of the autopsy coverslip preparations were made, as well as cultures in Esmarch roll-tubes in agar-agar, from the blood of the heart, from the lungs, liver, kidneys, spleen, a mesenteric lymph gland and the brain.

The examination of coverslips from several of these (heart's blood, liver and spleen) showed bacilli and oval forms, usually surrounded by a capsule, similar to those described in the hardened sections. All the cultures from the blood and various organs, after incubation for 18 hours, showed a luxuriant growth of colonies which had the same appearance in all the tubes. The same organism was found in pure culture in all the tubes.

Agar, plain or with glycerine or glucose. In cultures made in Esmarch tubes or in Petri dishes, after 18 to 24 hours in the incubator at 37° C., small, deep and larger superficial colonies appear. The larger colonies are from 1 to 2 or 3 mm. in diameter, raised above the surface of the medium, usually round in outline, and tending to pile up in the centre. They are thick, opaque, have a grayish white appearance, and tend to spread. Magnified 50 diameters the colonies are dark brownish yellow in the centre, and of a homogeneous appearance. The edges are lighter brown and somewhat irregular in outline. The deeper colonies are grayish white, opaque, and usually small. Those that reach the surface tend to pile up without spreading. Microscopically they are round, with clear-cut borders, dark brown in color and finely granular. After a few days these colonies, under the microscope by transmitted light, are darker, almost black in color, homogeneous, and somewhat oblong in shape. Streak cultures upon slants of plain agar show in 24 hours in the incubator and 48 hours at room temperature an abundant, luxuriant raised growth along the line of the needle. This growth is creamy white, polished, porcelain-like, standing well up above the surface of the medium, and usually with serrated edges. In a few days the growth spreads over the surface of the medium. The growth on glycerine agar and glucose agar has the same characters, but

is more luxuriant. Stab cultures in plain nutrient agar show after 18 to 24 hours in the incubator a feeble, white growth along the line of stab. Similar cultures in glycerine agar and glucose agar grow luxuriantly with the production in the latter of a large amount of gas. In old agar cultures the medium becomes brownish and there is an odor like that of stale glue. The growth is always viscid and sticky; gas bubbles are frequently seen in the water of condensation from glucose agar.

Blood serum.—The colonies on solidified blood serum are quite similar to those on agar. The growth is rapid and luxuriant, viscid and mucoid, pale, grayish-white, porcelain-like, raised, and tending to spread from the line of the inoculation. In a few days it covers nearly the whole of the surface. The water of condensation is diffusely clouded; the medium is not liquefied.

Gelatine.—At room temperature after 36 hours in plate or roll cultures there appear small, round, opaque, creamy white colonies 0.5 mm. in diameter. The centre of the colony is thicker and more elevated. Magnified 50 times, the colonies are of a yellowish color by transmitted light. Some of them tend to spread, but the majority have regular outlines. In some of the larger colonies the centre is umbilicated, while the peripheral portion is raised and arranged in concentric layers. The deep colonies are round with clear-cut margins, and of a dark brown almost black color when examined under the lower powers. The larger colonies when magnified 50 diameters are dark brown in the centre, fading to a dark brownish yellow homogeneous appearance at the periphery. The edges are light brown in color and rather irregular. Gelatine slant cultures after 24 hours at room temperature show a raised growth similar to that seen on agar and on blood serum, but less luxuriant. In gelatine stab cultures, along the line of the stab after 24 hours there appear small scattered grayish white colonies. At the surface of the culture is a small, raised, opaque, grayish white, round or oval growth, which after a few days tends to spread out over the surrounding surface. No well-marked, nail-shaped growth developed. Gelatine is not liquefied. In old cultures the medium sometimes becomes brownish. Gas production does not occur in plain nutrient gelatine.

Potato.—The bacillus grows luxuriantly upon potato both at room and body temperatures. In test-tube potato cultures the upper third of the growth does not usually spread from the line of inoculation, and is generally grayish white, sometimes dark brownish gray, dry and finely granular. The lower two-thirds of the culture nearly always spreads, so that here the whole surface and often the sides of the medium are

covered with a moist growth. This growth is sometimes raised and porcelain-like, and of a creamy color, sometimes simply smooth, moist and polished, without piling up. There is always gas production both upon the surface of the potato and in the fluid at the bottom of the tube.

Carrot.—The growth on this medium is like that on potato, but more luxuriant and with more marked gas formation.

Bouillon.—The growth in plain as well as in glucose and glycerine bouillon is rapid and luxuriant both at room temperature and in the incubator. After 24 hours' incubation the medium is diffusely cloudy. On the surface is a grayish white pellicle, adherent to the sides of the tube and usually covering the whole of the surface. At the bottom is an abundant, grayish white sediment, which on agitation breaks up into a thick stringy mass. In cultures several days old the medium becomes almost opaque, thick, tenacious and stringy like mucus. In bouillon containing glucose, saccharose, or any of the sugars there is abundant gas production. In the fermentation tube within 24 hours sufficient gas is formed from glucose bouillon to displace nearly all the medium in the upright arm.

Milk.—Milk is coagulated with gas formation and acid production, in from 24 to 36 hours in the incubator. The coagulum is hard and forms a solid mass surrounded by thin pale whey.

Dunham's peptone solution.—The bacillus grows rapidly in this medium and with the same characters as in bouillon. No indol reaction could be obtained.

The organism grows best in media of neutral or alkaline reaction, but will grow also on acid gelatine, potato and carrot. It grows best at 35°-37° C. It is killed by exposure for five minutes to a temperature of 60° C. It is non-motile and does not form spores. It is facultatively anaërobic, and stains well by both Gram's and Weigert's methods, as well as with the common aniline dyes.

General morphology of the bacillus. The morphological appearances of the bacillus are the same in the *blood* and *organs* of animals as in those of the human case. Coverslips made from the liver, spleen, kidneys and heart's blood showed bacilli often in pairs, varying from 0.5 to 3 or 4 μ in length, by 0.5 to 0.8 μ in thickness, generally with rounded ends. There were marked variations in size and shape. Many short oval forms were seen. Short forms resembling cocci and diplococci occurred, and occasionally long filaments were seen. The long forms often had square ends, while the shorter ones always had rounded ends or were ovoid in shape. Coverslips from the fresh blood or from the

organs always showed capsulated forms, the capsules frequently containing pairs. The shorter organisms were encapsulated more frequently than the larger ones. From the pleural and peritoneal exudates the organisms constantly showed capsules. Here the coccal, oval and short bacillary forms are the most common, long rods being unusual.

The capsules stain readily with carbolic fuchsin, with eosin and gentian violet, with or without aniline oil. The organism stains readily with all the usual dyes, and can be seen even in sections stained with eosin and hæmatoxylin. As previously stated, capsulated forms are rarely seen in sections of hardened tissues. In cultures there is the same polymorphism as that found in the human body. In fresh agar, glycerine agar, blood serum and gelatine cultures the most numerous forms are short, plump bacilli, with rounded ends; fewer coccal forms are seen, but there are often ovoid forms in pairs or short chains; some long stout bacilli are also seen. In young cultures, especially on glycerine-agar and on blood serum, capsulated forms are often seen. In fluid media the longer forms predominate, and many long filaments of from 5 to 8 or even 10 μ in length are seen. In older cultures on solid media the longer forms are numerous, and sometimes there are irregular swellings. On old potato or carrot cultures the predominating forms are long. Most of the longer forms stain poorly, and in many there are one, two, or even three deeply staining points, surrounded by somewhat granular, poorly staining protoplasm. In some there is an exquisite bipolar staining, while in others only one pole takes up the dye. Nothing suggestive of spores is to be seen.

Pathogenesis.—Cultures of the bacillus, both fresh and old, grown both on solid and in fluid media, both living and sterilized, were pathogenic for dogs, rabbits, guinea-pigs, white and gray rats, and house mice, while pigeons proved refractory.

Dogs.—Young dogs inoculated into the peritoneal cavity with 2 cc. of a 24-hour-old bouillon culture died in 36 hours with a thick purulent exudation in the peritoneal cavity, with marked congestion and ecchymoses of the peritoneum and congestion of the pericardium, pleuræ, liver, spleen, kidneys, stomach and intestine.

Rabbits.—Many rabbits were inoculated in the ear vein, subcutaneously, and into the peritoneal and pleural cavities, in doses varying from 0.2 cc. to 3 cc. of a 24-hour bouillon culture, or of a suspension in bouillon or salt solution of several loops of a blood-serum or agar culture. The smaller doses without exception were fatal, even for old, large animals, except when inoculated subcutaneously.

Intravenous inoculations killed rabbits in from 12 to 72 hours, the

animals dying of septicæmia with congestion of the various organs and serous membranes, with hæmorrhages in many instances, and sometimes with fibrino-purulent exudations. The spleen was not enlarged, and pneumonia was never found. Fatty degeneration of the liver, heart, and kidneys was commonly present.

Peritoneal inoculations killed in from 12 to 72 hours by septicæmia and peritonitis, often with hæmorrhages, and the formation of a stringy, mucoid exudate rich in short bacilli, both free and within polymorphonuclear leucocytes. On microscopical examination the veins and capillaries were found widely dilated, especially in the stomach, intestine, liver and kidneys. There were no focal areas of cell degeneration in any of the organs. The bacilli were abundant in the blood and organs.

Subcutaneous inoculations caused local purulent exudates, often followed by death from septicæmia with changes in the organs and vessels similar to those described above.

White *rats* died in from 24 to 48 hours after subcutaneous or intraperitoneal inoculation of 0.5 to 1 cc. of a bouillon culture. The organisms were always present in the heart's blood, in the various organs and in the peritoneal and pleural cavities. The same dilatation of the blood-vessels with focal hæmorrhages, as has been described in rabbits, was observed in these animals.

Wild gray rats died in from 18 to 24 hours, after either subcutaneous or intraperitoneal injection of small doses, with septicæmia and few changes in the organs.

House *mice* died on the fourth day after inoculation, whatever the mode of inoculation. There was seldom any local reaction at the point of inoculation, but septicæmia, with marked congestion of the vessels and multiple ecchymoses of the serous membranes and various organs, was present. The serous cavities contained an excess of fluid, which was thick, tenacious and stringy, and often contained polymorphonuclear leucocytes, endothelial cells, and red corpuscles, in addition to large numbers of bacilli. In the liver the most striking change was the enormous dilatation of veins and capillaries. The veins of both the portal and hepatic systems, particularly just beneath the capsule, were distended with blood and the liver cells were compressed and granular, and often contained large and small fat drops. Here and there were larger and smaller points of hæmorrhage. Many bacilli were seen in the capillaries and in the hæmorrhagic areas. Emboli of bacilli, however, were never seen. The lungs showed marked dilatation of the blood-vessels, in which bacteria, singly and in clumps, were present. Besides these, some unmistakable liver cells, granular and fatty, were detected in

the pulmonary arteries and capillaries. In the spleen, especially under the capsule, there were marked congestion and often hæmorrhage into the tissue. The red corpuscles in the tissue were in various stages of degeneration, and a large amount of yellow pigment was seen. In the kidneys the most marked change was hyperæmia, with here and there areas of hæmorrhage into the tubules and inter-tubular tissue. Bacilli were in the blood-vessels in large numbers, especially in the glomerular capillaries. They also appeared in masses in the tubules. The epithelium of the convoluted tubules was fatty. There were no focal areas of degeneration or of round-celled infiltration. In all the coats of the stomach and intestine there were congestion and hæmorrhages.

Guinea-pigs.—Peritoneal inoculations of from 0.2 to 0.5 cc. of a 24-hour-old bouillon culture caused death in from 12 to 48 hours. The inguinal and axillary glands were swollen and congested, with œdema of the periglandular tissue. There was always a peritoneal exudate, which in rapidly fatal cases was serous with few cells, and in animals living longer, thick and tenacious, with grayish opaque patches, containing many endothelial and polymorphonuclear cells. There was always congestion of and often hæmorrhages into the serous membranes. The heart was pale; the liver enlarged and deeply congested. Microscopically the same changes were found in the organs as in mice and rabbits. These were especially marked in the liver, where the central veins were often ruptured. The blood-vessels contained a large number of polymorphonuclear leucocytes and many bacteria, some of the capillaries being plugged with the latter. Here and there clumps of bacilli, surrounded by polymorphonuclear leucocytes, were seen in the tissues.

Subcutaneous inoculations in guinea-pigs were followed by abscess formation, œdema, and death from septicæmia after from 3 to 6 days; the autopsy showed the vascular and tissue lesions as in animals inoculated in other ways.

Pigeons were refractory to large doses administered either subcutaneously or peritoneally.

Bouillon cultures from two to six weeks old, sterilized by heat or by the addition of 0.5 per cent. trikresol, in doses from 0.25 to 0.5 cc. and over, killed rabbits, guinea-pigs and white rats; they were not pathogenic for dogs, but even in minute doses killed house mice. The bacteria-free filtrate of such cultures was also pathogenic for rabbits, guinea-pigs, and house mice in the same doses as the unfiltered cultures. The dead bacteria left in the filter even after repeated washings with water were pathogenic for the same animals.

An analysis of the results of experiments upon a large number of animals shows that the sterilized unfiltered cultures were the most toxic, the dead and washed bacteria stood next in toxicity, while the filtrate of the same cultures was least toxic. The animals inoculated into the ear vein or intraperitoneally with these substances became ill and died in from 6 to 18 hours. They showed marked congestion of the serous and mucous membranes with ecchymoses, congestion and hæmorrhage into the various organs. The liver cells and the epithelial cells of the kidney showed cloudy swelling and fatty degeneration, but there were no large areas of focal necrosis. The blood-vessels were widely distended, and there were marked hæmorrhages in the liver, spleen and kidneys. In the spleen there was found a large amount of yellow pigment, both intra- and extra-cellular. The pigment was usually in the shape of small granules, but larger masses were seen.

By starting with small non-fatal doses of both living and sterilized cultures and the filtrates of bouillon cultures, both guinea-pigs and rabbits could be accustomed to withstand doses fatal for untreated animals (1 to 1.5 cc.); but for these animals a dose of 2 cc. was fatal.

The blood serum of rabbits or guinea-pigs thus immunized from dead cultures and soluble toxines, even in large amounts (0.5 to 1 cc.), failed to protect fresh animals against the toxines or the dead or living cultures.

One large pregnant rabbit, receiving first small doses and then as much as 1.5 cc. of a sterilized bouillon culture on two occasions, carried her young to term. The mother, after gestation, became thin and her young were much smaller and grew much less rapidly than those of other rabbits born about the same time. Two of her young rabbits, when six weeks old, were inoculated with 0.5 cc. of a 24-hour bouillon culture of the bacillus, a fatal dose for a control rabbit of the same age but out of another mother. One of the two rabbits died within 24 hours; the other was apparently unaffected. 0.5 cc. of the blood serum of a third rabbit of this litter failed to protect one of another litter against a similar quantity of a 24-hour bouillon culture of the bacillus, but a large rabbit receiving 1 cc. of the same blood serum and 1 cc. of a sterilized bouillon culture of the bacillus did not die till the fifth day. From this it would seem probable that rabbits may inherit from the mother some degree of tolerance for this organism.

The bacillus above described has preserved its virulence and general biological characters during the five years since it was obtained

at autopsy. When compared by means of cultures and animal experiments with a number of capsulated bacilli of the type of Bacillus pneumoniæ of Friedländer,—including two examples of the bacillus of Friedländer obtained from cases of pneumonia, the capsulated bacillus of Pfeiffer, the bacillus of Wright and Mallory, and a capsulated bacillus obtained by Dr. Walter Reed, U. S. A., from the throat in a case of diphtheria, besides a number of capsulated bacilli obtained by the writer from inflammatory processes of the accessory sinuses of the nose, and from two cases of septicæmia, one following labor and the other pyelonephritis—the bacillus of the present case showed differences from them all. Of these differences the chief related to the varying amounts of gas and acid production, the capability and the rapidity of coagulation of milk, the behavior to Gram's stain, and the widely varying results of animal inoculation.

The bacilli above mentioned, as well as other allied forms, have been placed by Fricke (13) in a group of which the Friedländer bacillus is the prototype and to which E. Fraenkel gave the name "Bacillus mucosus capsulatus." The more important common characteristics of this group are the morphology—plump, medium-sized, pleomorphic rods; the presence of capsules, readily demonstrable in the animal body and sometimes in cultures; lack of motility and of spores; failure as a rule to stain by Gram; the rapid, luxuriant, elevated, viscid, white growth upon the surface of solid media; absence of liquefaction of gelatine; and pathogenicity, usually in the form of septicæmia, but with striking variations for different animals and for different members of the group. Fricke has collected from the literature 22 representatives of this group and to these has added from personal observations still others, which differ from each other more or less sharply by such points as minor cultural variations, particularly on potato, presence or absence of brownish discoloration of old gelatine or agar cultures and of a typical "nail-growth," the coagulation of milk, the occurrence of indol, production of gas and of acid, retention of Gram's stain, and, above all, susceptibility of different animals under varying methods of inoculation to the pathogenic effects of the organism. Opinion is divided, and it is at present indeed difficult to

decide, as to the value of these several points in the establishment of species or varieties. They have not hitherto sufficed for any satisfactory subdivision of the group into well-recognized species or varieties, although attempts to accomplish this have been made.

While the bacillus described in this paper cannot be identified with the bacillus of Friedländer and differs in certain respects from most, if not all, previously described members of the group, it nevertheless possesses the more important common characteristics of the group, and I therefore prefer, at least for the present, to place it here rather than to propose a new group. It is true that Kruse * emphasizes the absence of staining by Gram as one of the characters of his group of Bacillus aërogenes and bacillus of rhinoscleroma, under which group he classifies the various capsulated bacilli of the type of the bacillus of Friedländer, but the readiness with which the color can be extracted by Gram's method is certainly one of the variable attributes of the group and cannot serve as a decisive criterion in determining whether an organism belongs to the group or not.

Doubtless to the same group of capsulated bacilli (Bacillus mucosus capsulatus) belong bacilli isolated from human cases of hæmorrhagic infections by Bordoni-Uffreduzzi (6), Banti (5), Babès and Opresco (4), and von Dungern (10). We may, therefore, consider it established that members of this group of capsulated bacilli may cause typical hæmorrhagic septicæmia in man.

Different from the preceding are the bacilli cultivated from cases of septic purpura hæmorrhagica by Babès (1), Tizzoni and Giovannini (22), and Kolb (17). Babès classifies these bacilli together, although it is not certain that they are closely allied, and he considers that they have many points in common with Hueppe's hæmorrhagic septicæmia group. Their resemblance to Hueppe's group seems to me rather remote, but it must suffice here to call attention to these bacilli as distinct from the luxuriantly growing, capsulated bacilli already described.

It is clear that various bacteria, not only those already considered, but also streptococci and other bacteria, may cause hæmorrhagic infec-

* Flügge's Die Mikroorganismen, Th. II, p. 336; Leipzig, 1896.

tions in human beings. Hæmorrhagic septicæmia, then, is not to be regarded as a separate and distinct disease, with definite and constant etiology, uniform anatomical lesions and clinical features, as, for instance, typhoid fever, but rather as a toxæmia, attended by such marked injury to the blood-vessels and to the blood as to cause extreme dilatation and hæmorrhages, with various harmful effects upon the body cells, and capable of being caused by a variety of agents. The organic lesions differ widely in various cases. The similarity in the effects produced in animals and in man by the bacillus of my case is striking. In both, beside the vascular changes, fatty degeneration of the endothelial cells, and rupture with hæmorrhage into the tissues, there was marked destruction of the red corpuscles, with the accumulation of blood pigment in the liver, spleen and kidneys. Of interest also in my case is the direct and the retrograde embolism of the liver cells. The similarity of my cases to typhus fever is paralleled by the case reported by Babès and Opresco. A malignant, rapidly fatal course has characterized several of the reported cases, whereas others have been of milder type and longer duration. At least some of the instances reported as malignant purpura hæmorrhagica belong to the class of hæmorrhagic septicæmias.

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BACILLUS CAPSULATUS (BACILLUS PNEUMONIÆ OF FRIEDLAENDER?) WITH ESPECIAL REFERENCE TO ITS CONNECTION WITH ACUTE LOBAR PNEUMONIA.

A Report of Twelve Cases in which Bacillus Capsulatus Occurred in the Medical and Surgical Wards of the Boston City Hospital.

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Method. The method of investigation will be further described with the reports of cases. Löffler's blood serum slants were used as a routine procedure. Descriptions of growth, appearances of colonies, etc., refer to this medium unless otherwise mentioned. In addition to the blood serum the various solid and fluid media were used, as well as animal inoculations and various special staining methods.

REPORTS OF CASES.

The twelve cases in which the Bacillus capsulatus occurred were as follows: (These cases, with the one reported by Wright and Mallory (1) in 1895, represent all the cases in which the capsulated bacillus was observed in the Boston City Hospital during the past three years.)

Case I. Acute lobar pneumonia. The patient entered the hospital March 15, 1896, in the service of Dr. V. Y. Bowditch, with considerable dyspnœa and cyanosis and with signs of consolidation in the middle lobe of the right lung. Death occurred within 24 hours after admission.

Autopsy (Dr. Mallory), March 16. *Anatomical diagnosis: Acute fibrinous pneumonia of middle lobe of right lung, with extension into upper and lower lobes. Cloudy swelling of liver, kidneys and heart, and acute splenic tumor.*

Coverslips from consolidated lung at autopsy showed numerous large and small bacilli with rounded ends surrounded by a wide capsule, also many small lanceolate, encapsulated diplococci. The bacilli were completely decolorized by Gram, whereas the lanceolate cocci retained the stain.

Cultures from the hepatized right lung showed a profuse, confluent, colorless, stringy, mucoid growth with a few small, slightly opaque, pinhead colonies at the top of the culture medium. The profuse growth contained mostly large bacilli with rounded ends, surrounded by wide capsules containing from one to four rods, decolorizing slowly by Gram. The small colonies consisted of irregular groups of small cocci. The water of condensation contained both the large capsulated bacilli and many lanceolate diplococci.

From the liver there grew a number of elevated viscid colonies, varying in size from a pin's head to 5 mm. in diameter. These showed the same capsulated bacillus as in the lung.

The culture from the kidney contained 8 to 10 colonies similar to those from the liver. Cultures from the heart and spleen were negative.

Microscopical sections of the middle lobe showed distended alveoli filled with fibrin, red corpuscles, leucocytes and a moderate number of desquamated cells. By the Gram-Weigert stain many lancet-shaped diplococci were found in the alveolar and pleural exudates. In sections stained by Unna's alkaline methylene-blue were seen a few large and medium-sized bacilli in the alveolar exudate, and large numbers in the bronchi. In sections stained by the Gram-Weigert method no bacilli were observed.

The capsulated bacillus was completely decolorized in smears from the lung, in preparations from cultures, and in sections stained by the Gram-Weigert method, the decolorization in smears being considerably slower than in preparations from cultures.

Case II. Gangrene of the lung. Patient aged 68 years, a roofer by trade, was brought to the hospital in a moribund condition. From patient's brother it was learned that he had been sick for four weeks; there was considerable expectoration emitting a foul odor, and of a purulent character, sometimes containing blood. Physical examination showed evidences of a large cavity in an area about $2\frac{1}{2}$ inches in diameter just below the right clavicle. Patient died on the day following admission.

Autopsy by Dr. Mallory. Anatomical diagnosis: *General arteriosclerosis; gangrene of upper lobe of right lung, and acute vegetative endocarditis.*

Upon the mitral valve were fresh vegetations. In the right lung was a large cavity occupying almost the entire upper lobe, filled with a foul-smelling fluid, which contained small pieces of detached gangrenous lung. Other parts of this lung and the entire left lung were normal.

Coverslips from material in the pulmonary cavity showed many large and small capsulated bacilli, some cocci and a few slightly staining rods. Coverslips from the mitral vegetations showed a few medium-sized capsulated bacilli which decolorized by Gram's method.

Cultures from the upper lobe of the right lung presented a diffuse, translucent, stringy, mucoid growth of numerous small and large bacilli, some faintly staining rods without capsules, and a small number of cocci.

From the heart grew 10 to 12 viscid colonies, varying in size from a pin's head to 4 mm. in diameter, similar to those described in Case I.

The tube from the spleen showed 5 colonies, similar to those from the heart, and containing the same capsulated bacillus. Cultures from the liver and kidneys were sterile.

All the large colonies were composed of the capsulated bacillus. A guinea-pig, weighing 410 grms., inoculated in the peritoneal cavity with a pure culture on blood serum, died in 17 hours with general peritonitis and marked congestion of the liver and spleen. Cultures from the animal gave a profuse growth of the same organism as that from Case I.

Case III. Acute croupous pneumonia complicated with acute otitis media. The Bacillus capsulatus and the diphtheria bacillus were found in cultures made from the middle ear. The Micrococcus lanceolatus was found in the lung, heart's blood and kidney, but not in the middle ear in which only the capsulated bacillus and the diphtheria bacillus were demonstrated. There was no extension of inflammation from the ear into the mastoid sinuses or the cranial cavity.

Case IV. Fracture of base of skull accompanied by acute otitis media. The capsulated bacillus was found in pure culture in the middle ear, from which there was no extension of inflammation.

Case V. Diphtheria. In a young man, about 21, convalescing from diphtheria, the capsulated bacillus was found, with the diphtheria bacillus, in cultures from the throat. The capsulated bacillus persisted in daily cultures from the throat, until the patient was discharged nearly five weeks later. Diphtheria bacilli were present in small numbers for four weeks. Pure cultures of the diphtheria bacillus, isolated during four weeks, failed to kill guinea-pigs, even when injected in large quantities.

Case VI. After diphtheria. Culture from the throat showed diffuse growth of capsulated bacilli with a few streptococci, but no diphtheria bacilli.

Case VII. Diphtheria. Capsulated bacilli and diphtheria bacilli isolated in pure cultures. In the nose there were no capsulated bacilli

but many diphtheria bacilli. In the culture from the throat the capsulated bacilli grew so profusely that the diphtheria colonies could not be made out. The diphtheria bacilli in small numbers were seen only after smearing from the general surface of the serum tube in the water of condensation. The growth of the capsulated bacilli disappeared in three or four days, after which the diphtheria colonies grew luxuriantly.

Case VIII. Diphtheria. A few colonies of diphtheria bacilli and many large colonies of the capsulated bacillus.

Case IX. Tonsillitis and pharyngitis. Cultures showed a profuse growth of the capsulated bacillus and in the water of condensation a moderate number of streptococci. Patient ill three days.

Case X. Diphtheria. Cultures from the throat gave a profuse growth of capsulated bacilli and a few colonies of diphtheria bacilli. The cultures were examined daily for two weeks. The capsulated bacillus persisted up to the time of discharge. The diphtheria bacillus disappeared in a little over one week. The attack was very mild. The mucous membrane of the pharynx was congested, granular, and covered with a thick, glairy, slightly opaque, sticky material, resembling a diffuse growth of the capsulated bacillus on Löffler's blood serum. This material when touched with a platinum loop would draw out in long threads.

Case XI. Tonsillitis. Cultures from the throat gave a profuse growth of capsulated bacilli with a few chains of streptococci. The symptoms cleared up in a few days.

Case XII. Diphtheria. Cultures from the throat showed many capsulated bacilli and a few diphtheria bacilli.

Three other of the throat cases were inspected clinically by the writer and the throats showed the presence of a mucoid, glairy material similar to that in Case X. Of the foregoing twelve cases, the first four were seen by the writer only at the post-mortem examination. The other cases were observed clinically as well as studied bacteriologically. The six diphtheria cases were mild. The two instances of tonsillitis presented no unusual symptoms and recovered after a few days. In these eight cases, therefore, the severity of the disease was not increased by the presence of the capsulated bacillus.

In Case I the capsulated bacillus was found plentifully in the bronchi, while but few were present in the alveolar exudate. It evidently was not concerned in the pneumonic process. An acute endo-

carditis caused by the capsulated bacillus, as in Case II, is rare. The writer has been unable to find a report of a similar case in this country. Weichselbaum (2) in 1888 reported an instance of acute endocarditis due to a similar capsulated bacillus which he called Bacillus capsulatus endocarditidis.

In Case IV, in which the capsulated bacillus was found in pure culture in the middle ear there was no extension into the mastoid cells or the cranial cavity. Reports of cases of acute otitis media due to capsulated bacilli, while not uncommon abroad, especially in Germany, are not very frequent in this country. In Case III the capsulated bacillus was found with the diphtheria bacillus, and it is a question whether the former was responsible for the inflammation, as we have observed several cases of acute otitis media in this hospital due to the Klebs-Löffler bacillus alone.

It is evident from these cases that the Bacillus capsulatus, while not common in this country, is, nevertheless, not an extremely rare organism. Only in two cases (II and IV), did it seem to have any special pathological significance. Of the other two cases in which the bacillus was found at autopsy, in Case I there was a double infection and death was due to the pneumonia which was caused by the Micrococcus lanceolatus, and in Case III, death resulted from pneumonia and general infection with the Micrococcus lanceolatus.

DESCRIPTION OF THE BACILLUS.

The following description of the capsulated bacillus obtained from the lung of Case I answers for each of the twelve cases.

The bacillus is thick, of variable size, with rounded ends, on an average from two to three times as long as broad, enclosed in an oval wide capsule, and often united in rows of two, three or four elements within a single capsule. Sometimes it grows out into rods five or six times as long as broad. It stains with the usual aniline colors, but not by Gram, by which it is slowly decolorized. The capsule is constant both in tissues and in cultures. The capsule can be stained with any of the usual dyes when dilute acetic acid is used for washing out the excess of stain. The capsule is best demonstrated by the methods described under *capsule stain* (p. 175).

The capsulated bacilli isolated from the twelve cases are essentially identical. There are slight differences only in degrees of virulence, when inoculated subcutaneously into guinea-pigs. This bacillus is practically identical with that described by Wright and Mallory (1), the main difference being that Wright and Mallory's bacillus did not kill guinea-pigs by subcutaneous inoculation, whereas subcutaneous inoculation of guinea-pigs with our bacillus was fatal in from five to seven days, and after death the bacilli were found in each case in the heart's blood and the various organs.

Colonies on blood serum after 18 hours appear as slimy drops, transparent, round, elevated, with convex surface. The size varies from a pin's head up to 5 mm. in diameter. A confluent growth covering the entire surface of the medium is often seen. The colonies are thick and stringy, and when touched with a platinum loop draw out in long threads. The water of condensation is thick and of a whitish color. On 1 per cent. glucose agar slants the growth appears as a broad viscid transparent line. The water of condensation is thickened. Stab cultures in 1 per cent. glucose agar show gas formation at the bottom of the tube, and growth along the entire line of inoculation. In gelatine stabs, growth occurs along the entire needle track. At the point of inoculation there is an elevated mound-like knob or nail-head which is like that described by Friedländer (3). Bouillon becomes cloudy after 15 hours. The bouillon is thickened and viscid at the end of 24 hours. On potato there is a profuse, glairy, colorless, viscid growth. Milk is coagulated and acidified.

The organism kills white mice and rabbits when inoculated into the ear vein. In guinea-pigs intra-peritoneal injections kill in 24 hours, subcutaneous inoculation in from 5 to 7 days. At the autopsy there are found enlargement of lymph glands, a large soft spleen, the blood somewhat thickened, but not to the degree described by Pfeiffer (4) in his experiments with the capsulated bacillus. The adrenal glands of the guinea-pigs were hæmorrhagic as in the experiments of Wright and Mallory.

The organism here described is closely related to, if not identical with, the bacillus of Friedländer (3), and its description agrees closely

with that of the capsulated bacilli described by Wright and Mallory (1), Pfeiffer (4), Fasching (5), von Dungern (6), Mori (7), Mandry (8), Abel (9), Paulsen (10), Marchand (11). Loeb (12) and others have described capsulated bacilli differing from ours only in minor details and many of them are doubtless identical. Most of the studies of this group of bacilli have been made by foreign investigators. Of the varieties which most closely resemble ours, besides that of Wright and Mallory, may be mentioned those of Friedländer, of Pfeiffer, of Fasching and of Loeb. It is also probable that many described by others are identical and represent varieties of varying virulence. They certainly are all closely related.

Several attempts have been made to classify the various capsulated bacilli but without any great success. Wilde (13) has attempted to divide them into five groups, but his classification can scarcely be recommended. Fricke (14) has made a careful comparative study of the members of this group and has collated the characters of many reported in the literature.

Capsule stain. For staining the capsules both in cultures and in cover-glass preparations made from the organs a modification of Welch's method was used.

Welch's (15) method is as follows:

1. Cover the preparation (prepared without contact with water) with glacial acetic acid for a few seconds.

2. Drain off and replace (without washing in water) with aniline-gentian-violet solution. The staining solution is to be repeatedly added to the surface of the cover-glass until all of the acid is replaced.

3. Wash in aqueous solution of sodium chloride and examine in the same. The strength of the salt solution varies in different cases from 0.5 to 2 per cent.

This method depends upon the precipitation of the mucin-like substance of which the capsule is composed by the acetic acid, the precipitated material being insoluble in a 2 per cent. or sometimes weaker solution of sodium chloride.

I have found that by this method after using the salt solution, the specimen was often covered by a granular deeply staining detritus which often made it difficult to differentiate the capsule. The following modification was used by me and found satisfactory. It gives a much clearer picture and the capsules are stained more deeply.

1. Cover the preparation with glacial acetic acid for a few seconds.
2. Wash off the acetic acid with a 1 per cent. solution of potassium hydroxide.
3. Stain with aniline-gentian-violet for one minute without previously washing off the potassium solution.
4. Wash off excess of stain quickly in water.
5. Dry thoroughly with filter paper and over low flame and mount in balsam.

If the specimen is stained too deeply it may be decolorized by washing lightly in a 0.5 per cent. solution of acetic acid. The specimen should be completely dried before mounting in balsam, otherwise the bacilli will soon decolorize. This method is also well adapted for staining the capsules of the Micrococcus lanceolatus. I have coverslips prepared in this way which have not faded after two years. It may be added that while it is very easy to stain the capsules of the bacillus, it is often very difficult to stain those of the Micrococcus lanceolatus. If the slightest amount of water touches the specimen before the acetic acid is used the capsules are not stained but appear as clear halos around the cocci.

For staining the bacilli in sections Unna's strong alkaline methylene-blue, after the manner described by Mallory and Wright (16), was used. This stain is the most satisfactory to use for bacteria which decolorize by Gram and in connection with the Gram stain. Sections of lung from Case I showed very well the comparative numbers of bacilli and cocci.

RELATION OF THE CAPSULATED BACILLUS TO ACUTE LOBAR PNEUMONIA.

The history of the discoveries concerning the presence of bacteria in croupous pneumonia from the first observations of Klebs (17) in 1875 to the decisive papers of A. Fraenkel (18) and of Weichselbaum (19) in 1886 has been fully given by Welch (15) and need not be here repeated. Fraenkel came to the conclusion that the Micrococcus lanceolatus is the sole cause of genuine acute lobar pneumonia, whereas Weichselbaum, while recognizing this organism as the principal cause, claimed that about 5.5 per cent. of the cases of typical croupous pneumonia are referable to the Bacillus pneumoniæ of Friedländer. While the majority of investigators are probably of Fraenkel's opinion, not a few, especially the writers of text-books, hold Weichselbaum's view that a small percentage of cases of acute lobar pneumonia may be caused by the Friedländer bacillus and even

by other bacteria. Finkler (20), Honl (21) and Ziegler (22) may be cited as advocates of the latter view.

It has been our experience in the Boston City Hospital to find that true acute lobar pneumonia is invariably due to the Micrococcus lanceolatus. Pearce (23) in his report of 121 cases of acute lobar pneumonia which came to autopsy in this hospital from May, 1894, to May, 1897, found the Micrococcus lanceolatus in 118 or in 97½ per cent. of the entire number. The writer (24) reported in April, 1896, before the Boston City Hospital Medical Society, his investigations of 32 consecutive cases of acute lobar pneumonia. In every one the Micrococcus lanceolatus was found. Welch (15) found it in 10 consecutive cases at the Johns Hopkins Pathological Laboratory. Like results have been reported by many other observers.

It is an easy matter to overlook the Micrococcus lanceolatus, especially in mixed infections, as is illustrated by our Case I. In this case the cultures from the consolidated lung showed apparently on first inspection only the capsulated bacillus, but examination of sections stained by Gram-Weigert and careful examination of cultures with the application of the Gram stain revealed the presence of the Micrococcus lanceolatus. Undoubtedly many of Friedländer's cases were double infections, as he himself described the micrococci in the alveolar exudate and obtained the capsulated bacillus in cultures. It is of interest that Gram (25), working under Friedländer's direction, devised his stain for the purpose of demonstrating organisms in croupous pneumonia which he at the time believed to be identical with the capsulated bacteria obtained by Friedländer in culture, but which we now know to have been the genuine lanceolate diplococcus, this very stain being one of the most valuable means of differentiating these two bacterial species from each other. The occurrence of both these organisms in the same case is not very uncommon. This was so in Friedländer's series and appears to have been true for the cases of acute lobar pneumonia reported by Weichselbaum as due to the capsulated bacillus. The capsulated bacillus grows much more rapidly and profusely than the Micrococcus lanceolatus, thereby inhibiting the growth of the latter. It is well known that it is often difficult to

cultivate the Micrococcus lanceolatus, especially from old pneumonias, and that it will grow only on certain media. Inoculation experiments are often negative, even with pure cultures of the micrococcus. We cannot, therefore, always depend on procuring in cultures the Micrococcus lanceolatus from the solidified lung, especially in older cases, even when it is present. In a large percentage of cases there is a general infection with the lanceolate coccus. We have cultivated it repeatedly from the heart's blood and various organs, even when it did not appear in cultures from the lung.

It is probably due to the careful observance of the following rules that we have found the Micrococcus lanceolatus so regularly in acute lobar pneumonia:

1. Several cultures on blood serum are taken from the solidified lung, both from the older and the fresher areas, also cultures from the heart's blood, kidneys, liver and spleen.

2. Coverslip preparations are made from various parts of the solidified lung, also from the pleural and, if present, the pericardial exudates, at least 3 or 4 preparations being made from each location, and stained for capsules, and in such Cases as No. 1 by the Gram stain.

3. Sections of lung and of other organs, hardened in Zenker's fluid and in alcohol, are stained for histological study, and for bacteriological study both by Gram-Weigert and with methylene-blue.

4. Inoculations of animals are made when the results of the bacteriological examination at the autopsy are not decisive as to the presence of the Micrococcus lanceolatus.

Unless similarly complete examinations are made the absence of the lanceolate coccus cannot be accurately determined in cases of pneumonia. The reports of those investigators who have found instances of acute croupous pneumonia, which they have attributed to the capsulated bacillus, cannot, in my opinion, be accepted, as in none of them, so far as I have been able to determine, have the foregoing requirements been rigidly carried out. We are, therefore, as the result of our investigations, of the opinion that all instances of genuine acute lobar pneumonia are caused by the Micrococcus lanceolatus.

This investigation was conducted under the direction of Professor Councilman, to whom I am indebted for many suggestions and much assistance. I am indebted for valuable aid also to Mr. W. J. McDonald, student at the Harvard Medical School.

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REPORT OF EXPERIMENTAL WORK ON THE DILUTION METHOD OF IMMUNIZATION FROM RABIES.

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As is well known, the dried-cord method of immunizing human beings from the active principle of rabies was first worked out and used by Pasteur. Since his death (and even for a time before) little progress has been made in elucidating the still obscure pathology of the disease or toward the discovery of the special germ causing it. The dried-cord method of immunization is the one used in Paris and in other places where the treatment has been introduced and applied to human beings.

In examining the literature of the subject I was much interested in the investigations of Högyes* in Budapest, by whom a dilution method was employed in a series of experiments for the production of immunization from rabies. His hypothesis is that, contrary to the teaching of Pasteur and others, the dried cord contains a dilution pure and simple and not merely an attenuated virus. Therefore, if this supposition be true, a fresh dilution made, under aseptic precautions, from the medulla of a rabbit dead from rabies, would be more exact, require less time, and be less liable to produce infection by accidental contamination from extraneous organisms which in the older method might develop during the drying process. In Pasteur's method the

* Högyes, *Acad. des Sciences de Buda-Pest*, Oct. 17, 1887. *Centralbl. f. Bakter.*, 1887, ii, 579. Abstract with critical remarks by Roux in *Annales de l'Institut Pasteur*, 1888, ii, 94.

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cords are kept from three to fourteen days in glass jars or bottles at a temperature of 68° to 72° F. in dry air, and then emulsified before use. Emulsifying a cord is a long, tedious process, and considerable exposure of the material is necessary.

The dilution method does not rest upon a sufficiently satisfactory experimental basis and has not been developed enough to have secured its general adoption in the treatment of supposed infection by rabies in human beings. This method as used in Budapest is carried out in the following manner: A piece of the medulla weighing one gramme is taken from a rabbit just dead with rabies. To this is added 10 grammes of sterile broth and then with a glass rod it is beaten into an emulsion. This is the stock solution from which the dilutions are made, and is of the strength of one part of fresh medulla to ten parts of broth. The dilution used first is 1-10,000, then on successive days 1-8000, 1-6000, 1-5000, 1-2000, 1-1000, 1-500, 1-250, 1-200, 1-100, and finally the full strength (1-10) is given. The quantity of each solution injected subcutaneously is usually the same. The dilutions 1-10,000 to 1-6000 are supposed to correspond in strength to a cord dried by the Pasteur method from 14 to 8 days. Subdurally injected, they produce rabies only exceptionally; the dilution 1-5000 inoculated subdurally does not kill all rabbits and those killed succumb only after a protracted period of incubation; the dilutions 1-2000, 1-1000, 1-500, 1-250, always kill and after gradually lessening periods of incubation. The dilutions 1-100 and 1-10 act as quickly as the very thick emulsion made from the medulla used to produce rabies.

The advantages claimed for this method over the dried-cord method of Pasteur are, chiefly, much greater accuracy in giving a gradually increasing dose, and, secondarily, less chance of infection after removal of the cord from the body, as the stock solutions are made fresh each day and the dilutions are made from them, whereas in the Pasteur dried-cord method the cords are exposed to atmospheric changes for from 3 to 14 days before being used.

The work in which I have been engaged consists in modifying the dilution method by keeping one stock solution, from which the dilu-

tions are made daily, throughout the whole course of treatment, instead of making a new stock solution each day as is done in Budapest. The advantages of this procedure over the Budapest method I believe to be the following: (1) Avoidance of the tedious process of making an emulsion daily; (2) ability to test before use the stock solution for extraneous organisms, which may produce serious symptoms; (3) and most important (if this method eventually proves successful), the possibility of sending out from one centre, where the material is prepared, the stock solution, with directions regarding dilutions and doses. The physician in charge can then treat cases which perhaps are not able to travel a long way from home for treatment.

TECHNIQUE EMPLOYED IN PREPARING THE EMULSIONS AND GIVING THE SUBDURAL INOCULATIONS.

As the result of considerable experimental work carried on during the last eight months (the tables of a part of which are given below), I now make and preserve a stock solution in the following way: The brain of a rabbit dead from laboratory rabies (fixed virus) is beaten into an emulsion composed of 8 parts of brain and 80 parts of sterile glycerine and water. The amount of glycerine used is $\frac{1}{5}$ part of the whole emulsion; at first $\frac{2}{3}$ glycerine was employed, then $\frac{1}{2}$, then $\frac{1}{3}$, and finally $\frac{1}{5}$, which last has been entirely satisfactory. As Table I (p. 185) shows, larger amounts of glycerine than $\frac{1}{5}$ part diminish the virulence of the virus, increase the incubation period, and prolong the lives of the animals inoculated. The brain, after being thoroughly beaten up and emulsified by the aid of a glass rod, is then poured into a sterile cheese-cloth bag and filtered, in this way making a smooth mixture with no visible particles. To this emulsion $\frac{1}{5}$ part of glycerine is then added and thoroughly mixed. The mixture is then placed in a sterile flask. During these manipulations every possible precaution is taken to avoid bacterial contamination. The flask is placed in an ice-chest. This, then, is the stock glycerine solution from which all dilutions are made. Before being used, the solution is tested for extraneous germs. Experiments have shown that a stock solution may be kept in this way seven weeks with no diminution in its virulence. As a course of immunization treatment lasts about 2 to 4

weeks, there can be no question of the virulence of the stock solution remaining in full strength for that length of time.

In nearly all subdural operations chloroform was used as an anæsthetic with perhaps 2 deaths in 50 animals. A collodion dressing has been found serviceable as a protection to the wound, and since using it no abscess of the brain has formed, as had previously occurred in a few cases. Instead of holding the animal during subdural inoculations by tying it down on a board by each leg, I have substituted an apparatus which has been used with satisfaction. The reason for the change was that tying a rabbit or a guinea-pig to a board causes the animal to struggle and become frightened and prolongs the operation. The holder which I have devised is a round tin cylinder 10 inches long and 6 inches in diameter. Into one end of this fits a block of wood hollowed out on the inner end to fit the hind part of the animal. This block is pushed into the cylinder, according to the size of the animal. At the other end, fitting around the cylinder, is a movable bag-like arrangement which is provided on the free end with a draw-string. On each side of the bag-like arrangement are straps which fasten to the block of wood by nails. These straps are provided with a number of holes to provide for the size of the animal. The draw-string which fits around the animal's neck, is pulled fairly tight, and the ends are fastened on two nails on each side of the cradle which holds the cylinder in place. In this way the animal is held securely in a telescope-like apparatus. A smaller size is preferable for guinea-pigs.

The virus used in the laboratory came from Dr. Ruhräh of Baltimore, and has been passed through a series of about 180 animals until now the rabbits show beginning paralysis on the 5th to 6th day; marked paralysis on the 8th day; complete paralysis on the 9th day, and death on the 10th day. It has become, in other words, a "fixed virus." In guinea-pigs inoculated with this fresh virus, rabies appears on the 6th day and death on the 7th day after inoculation. These periods of incubation are very constant when this virus is used.

The experiments detailed in Table I were undertaken to test the effect of different proportions of glycerine on the incubation period

of this virus kept for 1-7 weeks in a glycerine solution. At first $\frac{2}{3}$ part of sterile glycerine was added to an emulsion of the fresh virus, then $\frac{1}{2}$ part, then $\frac{1}{3}$, and finally $\frac{1}{5}$. Rabbits and guinea-pigs were the animals used for the experiments.

TABLE I.

EFFECTS OF DIFFERENT PROPORTIONS OF GLYCERINE ON DURATION OF INCUBATION PERIOD.

Proportion of Glycerine.	No. of Experiments.	Kind of animal used.	Date of preparation of virus.	Time solution was kept.	Effect on incubation period.
$\frac{2}{3}$ part.	3	Guinea-pigs.	July 7.	9 days.	Increased 2 days.
$\frac{1}{2}$ part.	3	Guinea-pigs.	July 15.	8 days.	Increased 1 day.
$\frac{1}{3}$ part.	Not sufficient to tabulate.				
$\frac{1}{5}$ part.	1	Guinea-pig.	Oct. 5.	7 days.	Not increased.
$\frac{1}{5}$ part.	1	Rabbit.	Oct. 5.	10 days.	Not increased.
$\frac{1}{5}$ part.	4	Rabbits.	Oct. 13.	7 weeks.	Not increased.
$\frac{1}{5}$ part.	2	Rabbits.	Oct. 25.	5 weeks.	Not increased.

Examination of Table I shows plainly that the proportion of $\frac{1}{5}$ sterile glycerine in the stock solution does not diminish the virulence of the solution during a period of seven weeks, rabies being produced at the end of this time with the same incubation period as that produced by an emulsion freshly made. This is an important point, because it shows that one stock solution may be kept through a whole course of treatment, which never lasts longer than 2 to 4 weeks. Many more experiments were made to test the virulence of solutions kept in this way, and made with dilutions from a stock solution; but the results simply confirmed those given and they need not therefore be repeated.

EXPERIMENTS IN IMMUNIZING ANIMALS FROM RABIES.

The principle of the dilution method is that inoculations of a very small amount of virulent material do not produce rabies, and that the gradual increase of the amounts injected accustom the animal to the most virulent material. As the stock solution is represented by one

part of medulla to 10 parts of sterile water and glycerine, we should in the immunization treatment take a small part of this and dilute it to 1-10,000 for the first treatment, giving part of a cubic centimetre subcutaneously to each animal as a first dose. For the second injection, the dilution is diminished to 1-9000, then to 1-8000, and so on, lessening the dilution each day. In Budapest the injections are begun with a dilution of 1-10,000 and the strength rapidly increased within the period of two weeks to a dilution of 1-10. In the first few series of experiments on immunization of animals I followed the scheme of dilutions and doses as described by the investigators in Budapest; but I now believe that the dilutions should be as follows: 1st day, 1-10,000; then upon successive days 1-9000, 1-8000, 1-7000, 1-6000, 1-5000, 1-4000, 1-3000, 1-2000, 1-1000, 1-900, 1-800, 1-700, 1-600, 1-500, 1-400, 1-300, 1-200, and then this last dilution employed until treatment has been continued 2 to 4 weeks. Any dilution below 1-200 is likely to produce rabies. The dose of these various dilutions should be proportioned to the size of the animal. It should be noted that guinea-pigs are very susceptible to the virus of rabies, apparently much more so than rabbits. After finishing the immunization treatment in each series of animals, a subdural inoculation of fresh virus has been made in nearly all the cases, as a test of immunity. These inoculations have been made in some animals immediately at the end of immunization and in others within 2 weeks of its cessation. A guinea-pig or rabbit subdurally inoculated with fresh laboratory virus invariably dies, unless previously immunized, as has been proven in a large number of experiments. If, therefore, after a subdural inoculation with fresh laboratory virus the animal does not die within 10 days, we may feel sure that immunity has been secured by the treatment given. The subdural inoculation test is the most severe we can employ for testing the immunity of an animal.

Table II shows the results in different series of animals treated by the glycerine dilution method to produce immunity from rabies. In the first experiment, two to three stock solutions were used during the course of the treatment and dilutions made from them. In the last two series, one stock solution has been kept throughout and the

daily dilutions made from it, beginning with a dilution of 1-10,000, and then gradually increasing the strength as described above until 1-200 was reached in the case of the guinea-pigs and 1-100 in the case of rabbits.

TABLE II.

RESULTS OF IMMUNIZING TREATMENT BY DILUTION METHOD.

Number of animals in series.	Duration of treatment.	Highest and lowest dilutions used.	Animals died rabid during treatment.	Died from other causes.	Immune from effects of subdural inoculations after treatment.
A. 4 guinea-pigs	14 days	1–10,000 1–10	None	None.	None.
B. 6 rabbits	4 weeks	1–10,000 1–10	None	5*	1
C. 9 guinea-pigs	3 weeks	1–10,000 1–10	5	1	1†
D. 40 guinea-pigs	20, 3 weeks 20, 4 weeks	1–6,000 1–10	20	2 1 lost	17‡
E. 6 rabbits	3, 3 weeks 1, 4 weeks	1–10,000 1–100	None	1 injured 1 lost	1
F. 50 guinea-pigs	20, 3½ weeks 30, 4 weeks	1–10,000 1–200	None	3 suddenly 2 injured	20§

In the final inoculations to test immunity control animals were inoculated in the same way as the treated animals, and all died from rabies in 7 to 10 days. It will readily be seen from Table II that, while immunity can be produced by this method of treatment, in several instances rabies was produced by the treatment. This result, in my opinion, annuls any advantages this method may otherwise have over the dried-cord method of Pasteur.

The foregoing results were obtained during the year 1897 and the investigations were continued during 1898, with both the dilution and the dried-cord methods. I have had good opportunity to test the dried-cord method of Pasteur during the past eleven months.

The Health Department of New York City has adopted the Pas-

* This series was treated during the heat of the summer when unused animals in the laboratory died in large numbers from no apparent cause. Inoculations made from these animals showed no evidence of rabies.

† The other two animals in this series received subcutaneous inoculations with fresh virus, and lived.

‡ All the animals which lived through the treatment were immune from effects of subdural inoculations.

§ 25 animals were not immune from effects of subdural inoculation.

teur antirabic treatment and during the past eleven months I have treated thirteen cases by the Pasteur method, the first six in conjunction with Dr. Robert J. Wilson. Besides the treatment of human beings experiments upon animals have been conducted in order to test the Pasteur method and various modifications of it. The results have been in general confirmatory of those reported from the Pasteur Institute in Paris. In order to make sure of the absence of contamination from the inoculated material we have not only tested the cords before using, but have also tested the emulsion of the cord after it had been prepared and have awaited for 18 to 24 hours the results of the bacteriological test, the emulsions during this period being kept on ice, a procedure which can be followed without any impairment of the virulence of the emulsion, as we have proven experimentally. Besides these precautions, animals can be simultaneously treated with the same material as that given to the persons under treatment. Under these precautions we have observed no local or general disturbance whatever after the injections. In four instances the emulsions of the dried cords made each day were sent by a special messenger out of town packed in ice and were used with perfectly satisfactory results. We have thus secured by the dried-cord method the special advantages claimed for the dilution method without the dangers of the latter.

CONCLUSIONS.

1. I have simplified the dilution method by using a stock glycerine emulsion of the virulent cord, from which the desired dilutions can be readily prepared. The proportion of glycerine should not exceed $\frac{1}{5}$ part, if it is desired to retain the full virulence of the emulsion.

2. There is some danger of giving rabies to animals in the dilution immunization treatment, a danger which is not present in the Pasteur method.

3. The dried-cord method does not rest solely upon the principle of dilution, but is based also upon attenuation of the virus.

4. The Pasteur method being entirely free from the element of danger which pertains to the glycerine dilution method and resting upon a sounder experimental basis is the one to be preferred.

A STUDY OF THE SPINAL CORD BY NISSL'S METHOD IN TYPHOID FEVER AND IN EXPERIMENTAL INFECTION WITH THE TYPHOID BACILLUS.*

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Plates II–IV.

This paper presents the results of the study of certain changes noted by means of Nissl's staining method in three cases of typhoid fever and in a series of experimental inoculations of rabbits with Bacillus typhosus. My observations relate mainly to the alterations shown by this method in the motor cells of the ventral horns of the spinal cord and in the nerve cells of the dorsal root ganglia. With the Nissl method only one constituent of the cell body is stained by the basic dye, and hence this is called the stainable or chromatic substance. In the normal nerve cell of the motor type this consists of coarse, spindle-shaped masses which are regularly distributed throughout the body of the cell, and, with their long axes more or less parallel, run from process to process. On closer examination these masses, called the "Nissl bodies," are seen to be composed of an aggregation of small deeply staining granules (Held, 14). Into the dendrites these masses are continued less thickly distributed and drawn out into thin threads, plastered against the wall or angle of some branch, or coursing through the middle of the process. The axone on the other hand is entirely clear of stained particles, as is also a small area about its origin, known as the "axone hillock." The nucleus occupies the centre of the cell and, with the exception of the nucleolus, contains normally no substance stainable by Nissl's method. The nucleolus is small, sharply outlined and very intensely stained.

The points to be emphasized, as regards the normal cell, as seen by

* Received for publication April, 1898.

this method, are the fairly constant size of cell body, nucleus and nucleolus, the even, regular distribution and distinct character of the Nissl bodies, the central position of the nucleus, the clear nuclear substance and the small deeply staining nucleolus.

The stainable constituent of the cell body is now generally believed to represent a nutritive substance, previously soluble in the fluid portion of the cell, but precipitated by fixing agents as incrustations upon the achromatic or unstained portion. The achromatic portion of the body of the cell is regarded by Marinesco (22, etc.) as an unstained network prolonged into the processes as minute fibrils, which represent the conducting portion of the cell. Van Gehuchten (9) would account for the regular shape and arrangement of the chromatic bodies by supposing them to be incrustations, especially at the points of intersection of this network.

It obviously makes no difference in the results of our study of pathological effects whether we believe these structures to exist definitely as such in the cell during life, or to appear only after death as the result of fixing agents. What is of importance is that they possess in the normal cell a definite arrangement, constant for a variety of fixing agents and stains, though best shown by the Nissl method (Flatau, 6).

The normal cell shows variation from this type chiefly in the amount of chromatic substance. When this is present in larger amounts, the condition has been termed by Nissl (29, 30) "pyknomorphism," and has been thought to represent a state of rest. When, on the other hand, the nutritive element has been used up and chromatic substance is scanty, he has termed the condition "apyknomorphism." This variation in amount of chromatic substance does not alter the regular shape or regular distribution of the Nissl bodies. When these are found broken apart and destroyed, the condition is pathological, and for it Marinesco (21) has suggested the term "chromatolysis."

METHODS.

In the cases of typhoid fever to be described sections were taken from the cervical, thoracic, and lumbar regions of the cord and their respective dorsal root ganglia when practicable. Small pieces of the tissue

were placed at once in 95 per cent. alcohol and allowed to remain there from 12 to 20 hours. They were then transferred to absolute alcohol, and thence to a mixture of equal parts of absolute alcohol and xylol, remaining in each 12 to 20 hours, the longer period being more desirable, as it produces less distortion. After 5 hours in xylol they were kept in melted paraffin at about 55° C. for from 3 to 4 hours, and then embedded in paraffin having a melting point of 52° C.

The sections were stained in Nissl's methylene-blue solution, and differentiated in 0.1 per cent. alum solution, as recommended by Held (14). For mounting benzine colophonium was used, the benzine being driven off with as little heat as possible.

THE HUMAN CASES. SPINAL CORD.

Of the three human cases the first was of ordinary character, but a very severe infection; the second a severe infection ending in perforation and sudden death; the third a very severe infection in an old man, unrecognized before autopsy. The experimental material was derived exclusively from rabbits.

Case I. Man, aged 28 years, who died in the hospital after a severe attack lasting 11 days from date of onset. The ordinary signs of typhoid fever were present, and there were no complications except unusual nervousness and delirium, and a short time before death, difficulty in swallowing, which may be accounted for by ulcers in the œsophagus found at autopsy.

The autopsy was made 6 hours after death, the body having been preserved on ice. In addition to intestinal ulceration, ulcers were found in the gall bladder, stomach, œsophagus, larynx, and on the tongue.

The bacteriological examination showed pure cultures of Bacillus typhosus in the liver, spleen, mesenteric and retroperitoneal lymph glands, and urinary and gall bladders. The ulcers of the œsophagus and larynx contained streptococci and staphylococci.

The number of altered nerve cells in the cord is very numerous, but in most of these the alterations are not marked, except in the lumbar region of the cord. The size of both cell and dendrites is increased; in the more advanced stages the cell body is considerably enlarged. In the cell there has been a breaking apart and partial or complete solution of the chromatic substance or the Nissl bodies,

advancing from the region of the nucleus out toward the periphery. The component granules of these bodies not merely are separated and partly destroyed but also appear more or less diffused in the fluids of the cell, so as to form when precipitated pale, blurred flocculent-looking masses. [Plate II, Fig. 1.]

This process has not gone on to the same extent in all cells. Some, notably in the cervical and thoracic regions, vary hardly at all from the normal type. Others, and these in great numbers in the lumbar region, show an advanced stage of this form of chromatolysis. In this region wherever the axone with its hillock is accurately determined, the dissolution of the granules is especially marked in that portion of the cell, and, wherever pronounced, the nucleus has migrated somewhat toward the opposite side of the cell. In these cells the slightly enlarged dendrites are almost free from chromatic substance, rendering the determination of the axone, although not a more difficult problem, yet one requiring more care. [Plate II, Figs. 1 and 2.]

The achromatic substance has been altered in some parts but very slightly, and in such a way as to stain very faintly with the methylene-blue, a condition which, with the absence of the chromatic substance, serves to show more clearly its structure. In the dendrites and body of the cell, this staining is of a more indefinite nature; about the axone hillock, the staining is granular or net-like; in the axone, in the form of very few and fine, parallel, granular streaks. (Marinesco, 22.)

The nucleus is not enlarged in the cells of less advanced chromatolysis. Under the low power in the nuclei of most of these cells there is apparently a diffuse blue stain. This, under the high power, resolves itself into minute, faintly staining granules. In the more advanced stages the nucleus, in addition to being often eccentric, has become considerably enlarged, while the granular deposit is not increased and therefore is less thickly and more unevenly distributed within it. In all stages the nucleolus is greatly enlarged and less deeply stained than normal. In many of the cells it is so faintly stained and so vacuolated as to be scarcely visible, while in others it is undergoing complete disintegration.

Case II. Woman brought to the hospital suffering from typical typhoid fever. The disease ran an ordinary course until the morning of the 10th day, when the patient sank rapidly with profuse sweating, vomiting, dull delirium, distended and tender abdomen and died at 7.30 that night. There was a leucocytosis of 22,600.

At autopsy, 14 hours after death, a perforation of the ileum, with a consequent general, sero-fibrinous peritonitis, was noted in addition to the usual post-mortem appearances of typhoid fever.

Typhoid bacilli were cultivated from the bile, spleen, mesenteric glands and kidney; Bacillus coli from the lung, peritoneum, kidney, spleen, and liver.

Obviously the secondary infection of the peritoneum makes this not an absolutely pure case, but in view of the long period of infection with the typhoid organism and the shortness of the secondary infection, the latter can be practically disregarded.

The changes in the ventral horn cells are so nearly like those of Case I that they may be dismissed with a few words. In the numbers of the cells affected and the characters of the degeneration the two cases are nearly identical. The only difference is in a slighter degree of alteration in the nucleoli, these retaining in Case II a deeper stain and more definite outline. In some cells, although the chromatolysis spreads inward from the axone hillock, there are a few large abnormal blocks of chromatic substance filling in the corners formed by the entrance of the axone and the periphery of the cell.

In the two foregoing cases, in spite of the great and early disappearance of chromatic substance from the dendrites, by far the greater number of cells show the central form of chromatolysis. The point of entrance of the dendrites is in nearly all cases blocked by the remains of Nissl bodies, which had been altered much less than those in the more central portion of the cell [Plate II, Fig. 1]. There are a few cells, however, which show a more complete destruction advancing from the periphery and in which the nuclei are not eccentric. It was noted, however, that this appearance is, in some cases, in sections which do not show the nucleus, but have passed through the dendrites at the periphery of the cell, and that, in the succeeding section or sections, as one approaches the nucleus, this appearance gives place to the central form of chromatolysis. In cells showing this peripheral

character of chromatolysis the nucleus when present in the section is not eccentric, but it seems to be undergoing disintegration in the centre of the cell.

Case III. Man, aged 67 years, who entered the hospital in a dull, listless state, soon passing to unconsciousness, and from whom no satisfactory replies could be obtained. Friends stated that he had been ailing for two months with loss of appetite and weight. For two weeks before admission he had pains in the back, indigestion and shortness of breath and was very thirsty. Physical examination showed a large area of dulness in the right lower lobe with friction and tubular breathing. There was no expectoration. There was a leucocytosis of 15,000. The pulse was 128, while the temperature rose toward evening to 104° F. These facts naturally led to the diagnosis of senile pneumonia. The patient died two days after admission, and the autopsy was made within six hours after death.

At autopsy there was found a recent thrombus in the branch of the pulmonary artery supplying the lower lobe of the right lung, and consolidation of this lobe with gangrene and perforation of the visceral pleura. The spleen was enlarged and soft, but no intestinal lesions were present. The bacteriological examination, however, revealed the presence of Bacillus typhosus in the consolidated area in the lung, in the spleen, and in other organs. This case, which will be reported in detail elsewhere, is of especial interest as an example of infection with the typhoid bacillus without intestinal lesions.

Unfortunately the cord in this case could not be removed in its entirety, and therefore only as much as could be taken out through the cranial cavity could be reserved for examination.

In this case the earlier alterations described in the first two cases can be traced in only a few cells. The vast majority of cells show a much more advanced stage of alteration. These cells are swollen and distorted in varying degrees. The central portion of the cell is a mass of ill-defined, extremely pale, flocculent-looking material, studded everywhere with small round refractive bodies, probably representing remains of the normal achromatic network [Plate II, Figs. 3, 4 and 5]. Scattered along the periphery are a few small and large clumps of pale washed-out looking granules undergoing the later stages of disintegration and solution.

The nucleus is large and swollen, and sprinkled with the finely granular deposit. It no longer occupies a position anywhere near the centre of the cell, but bulges from the periphery. Along its internal margin there is often a fine dark line composed of minute deeply staining granules [Plate II, Figs. 3 and 4]. The nucleolus is pale and vacuolated and frequently may be seen to protrude from the prominent nucleus. In some few cells there seems to have been an actual extrusion, the nucleolus lying free with the remains of the crumpled nuclear membrane about it [Plate II, Fig. 4]. This latter appearance, however, may be due to tearing with the knife, although no injury to the surrounding tissue can be made out.

There seems to be a marked disappearance of many processes from the cells, perhaps an illusion due to the great increase in size.

THE DORSAL ROOT GANGLIA IN HUMAN CASES.

The consideration of the dorsal root ganglia in these cases, as elsewhere, is attended with certain difficulties. These are, chiefly, the absence of processes, and the great variation under normal conditions in the size of the cells and in the amount and distribution of the chromatic substance. Thus Lugaro has divided these cells into five normal types. For this reason it has been thought better to notice only such alterations as are well marked, and to discard all those of a doubtful nature. Owing to the refusal of permission to remove the cord in the third case the ganglia could not be studied. The following statements, therefore, are confined to Cases I and II, that is, to the ones showing less alteration in the cells of the ventral horns.

The alterations of a definite nature are identical in both cases and present in only a few cells. They consist mostly in a rarefaction of the chromatic substance and extremely eccentric position and even bulging of the nucleus. A mass of chromatic substance is often plastered against the cellular margin of the nucleus, which, though large, is much shrivelled, often full of a granular deposit, and contains a very large pale vacuolated nucleolus [Plate III, Fig. 12]. In addition a small number of cells show absence of chromatic substance in a central zone between a ring of large globular-looking bodies about the

periphery, and a collection of similar bodies immediately about the nucleus (as in Rabbit VI, Plate IV, Fig. 16).

EXPERIMENTAL CASES. SPINAL CORD.

In order to determine the constancy and mode of progress of the previously described lesions in typhoid infections the following inoculatory experiments were made on rabbits:

Inoculations were made into the ear vein, using bouillon cultures of the typhoid bacillus derived from the second human case. The following table shows the character of each experiment:

TABLE

SHOWING HISTORY OF INOCULATED RABBITS.

Number of Animal.	Dose.	Remarks.
I.	2 cc. 80-hr. culture.	Very intense reaction. Died in **2 hours.**
II.	2 cc. 70-hr. culture.	Ordinary reaction. Killed in **7 hours.**
III.	2 cc. 96-hr. culture.	Ordinary reaction. Killed in **33 hours.**
IV.	1st day, 2 cc. 2-day culture. 3d day, 2 cc. 5-day culture.	Ordinary reaction after first dose. After second dose animal rapidly succumbed in a few hours, *i. e.* **52 hours** after first inoculation.
V.	1st day, 2 cc. 2-day culture. 3d day, 2 cc. 5-day culture.	Ordinary reaction after first dose. After second dose animal extremely weak; lost weight for several days. At end of first week seemed very feeble in hind legs. As symptoms of convalescence began to appear, animal was killed **9 days** after first inoculation.
VI.	First 5 days, 1 cc. 6th and 7th days, 2 cc. 8th day, 15 cc. into peritoneum.	Animal very resistant. Blood agglutinated rapidly at end of 7th day. On 8th day 15 cc. were injected into peritoneal cavity without effect. On 23d day paralysis of hind legs first noted. Animal killed and autopsy made **24th day** after first inoculation.
VII.	2 cc. 72-hr. culture.	Animal passed through first effects and was allowed to live. About 29th day paraplegia of hind legs first noted; great emaciation of rump and legs. As symptoms of recovery set in, animal was killed on **64th day** after first inoculation.

As will be seen by consulting the table an attempt was made to get the degrees of toxic effect at different periods.

In *Rabbit I*, 2 hours after inoculation, the changes though noticeable are slight and confined to a few cells. They consist of slight swelling of the Nissl bodies and a tendency to disintegration and disturbance of their orderly arrangement.

In *Rabbit II* these alterations, already in 7 hours, have become marked, and notably so in the lumbar region of the cord. They correspond in nature, if not in degree, to those already described in the first and second human cases, and consist, as before, in the pale washed-out form of chromatolysis starting from the axone hillock and displacing the nucleus slightly to the opposite side [Plate III, Fig. 7]. The nucleus shows slight enlargement and the finely granular deposit. The nucleolus is large, pale and vacuolated. The enlargement of the cell and dendrites, the disappearance of chromatic substance from the latter, and the alterations in the achromatic substance are not pronounced.

In *Rabbit III*, which had been subjected to infection for the still longer period of 33 hours, the number of normal cells is greatly reduced, and it becomes evident at once that we have to deal with two different kinds of alteration. Most of the altered cells present the same appearance of central disintegration and solution of the granules spreading from the axone hillock, with the accompanying changes in nucleus and nucleolus, already described in the human cases and the second animal.

An appreciable number of cells, however, present the type of peripheral destruction observed in a few cells of the first two human cases. In some of these the cell body and processes are somewhat contracted and their outline is no longer regular. In the periphery of the cell any distinction between chromatic and achromatic portion is almost lost, the whole substance appearing granular and shred-like, with here and there small chains of disintegrated granules [Plate II, Fig. 6]. Toward the centre of the cell these chromatic masses become more and more abundant, until about the remains of the nucleus they are quite densely packed. The appearance in some of these cells is as if, progressing from the periphery, the Nissl bodies had been broken apart and destroyed *in situ*, not washed out into the surround-

ing substance. The nuclear membrane has disappeared, and the only sign of the position of the nucleus is a slightly rarefied area containing a dark, disintegrating nucleolus.

In some of these cells single or multiple clear areas resembling vacuoles may be observed [Plate II, Fig. 6]. These correspond, presumably, to those described by many observers in a variety of experimental intoxications, and seen by Babes (1) in typhoid and in other experimental infections to contain the organism used for inoculation. If present in large numbers such appearances may be significant. In my study this appearance was observed in one cell only, and in this animal, and is depicted in Plate II, Fig. 6. Other cells of this type show very little irregularity of outline or contraction, while the achromatic substance does not seem much altered. The granules are paler and more washed-out looking, though more numerous and in larger groups about the nucleus, which also is not much altered.

The whole gray matter of the cord of this rabbit is infiltrated with leucocytes, mainly small mononuclears, which are very abundant in the small dilated capillaries, in which they may be seen in the act of migration, and thus to invade the pericellular lymph spaces.

In *Rabbit IV*, which succumbed after a period of 52 hours, nearly all the cells are affected; practically no normal cells can be found. The type of chromatolysis which begins at the periphery, however, has almost disappeared, while the other or central form has become very prominent. The former lesion, that characterized by peripheral destruction, in addition to affecting fewer cells than in Rabbit III, differs also in the absence of any greatly contracted cells and of the appearance of vacuolization described in the foregoing animal. The cells resemble the other variety of this type. They are perhaps slightly enlarged, pale, diffusely blue, with a few flakes of chromatic substance grouped about the nucleus, which is little altered.

The latter type of lesion, that characterized by central chromatolysis, in addition to affecting a greater number of cells than before, has advanced considerably in the individual cells. These are large and swollen as well as their dendrites, which are nearly free from chromatic substance. At times one of two dendrites, which join to enter

the same cell, is almost entirely deprived of chromatic substance, while the other contains more or less well-formed Nissl bodies, a difference which exists even after their juncture, and before the cell body proper is reached. The disintegration and washing out of the granules has progressed pretty thoroughly throughout the cell, leaving but a faintly marked zone of slightly larger collections of granular debris at the periphery. Where the axone is cut this alteration as before may be seen to extend outward from the axone hillock.

The nuclei are only slightly eccentric, and show the same general appearance of a deposit, grouped especially about an irregularly swollen nucleolus [Plate III, Fig. 8].

Rabbit V lived for 9 days after the first inoculation and during this time lost much weight and nearly all control of the hind legs. The animal was killed as symptoms of recovery began to appear. The ventral horn cells in the lumbar region showed, contrary to expectation, very few cells similar to those already described, although great care was taken to cut in sections all parts of this region. Nevertheless, in that portion just above the entrance of the sciatic nerve, groups of large swollen cells were found with slightly eccentric nuclei, but in which Nissl bodies of characteristic shape and distribution were arranged somewhat concentrically about the nucleus, with a slight intensification of single granules in that region. These granules are bright and freshly staining, and their arrangement is very regular [Plate III, Fig. 10]. From their position in the cord these cells probably send fibres into the sciatic nerve. Alterations found in this nerve and the large size of these cells seem to indicate that they must have undergone previously some change and probably that of the central type already described [Plate III, Fig. 8]. The newly formed, regular appearance of the Nissl bodies, taken in consideration with the fact that this animal was recovering, suggests strongly the idea that these cells are undergoing a process of restitution. There is no infiltration with round cells demonstrable.

Rabbit VI proved very resistant to repeated inoculations and was allowed to live. There developed gradually paralysis of both hind legs, which was first noted on the 23d day. The emaciation of the

muscles of the rump and legs was very marked. On the 24th day the animal was killed and autopsied. The alterations found in this case are of interest both on account of their character and their distribution.

In the cervical cord there are scarcely any pathological changes. The cells, however, are in an apyknomorphic state, that is, the chromatic material seems to have been used up to a considerable extent, but the normal arrangement still remains. There are, moreover, some cells which are larger than others and present a slight suggestion of chromatolysis about the axone hillock and in the dendrites.

In the thoracic portion of the cord there is nothing of note.

In the lumbar enlargement most of the cells are of the normal type, and, in contrast to those of the cervical portion, are in an extreme state of pyknomorphism. The Nissl bodies are very numerous, in many cells slightly smaller than usual, and connected by small threads of granules forming a dense network of chromatic substance. A very few cells are much enlarged, and the chromatic substance is disintegrated and undergoing dissolution pretty evenly throughout the cell. The nuclei and nucleoli in these, as well as in some of the cells of more normal type, present the same general changes as those described in the previous cases.

But in the immediately adjacent portion of the lumbar enlargement, just above the entrance of the sciatic nerve, there is a localization of marked alterations, corresponding, it may be assumed, to the area of nervous control of the paralyzed muscles. A number of cells are still normal or only slightly changed, but here and there mere shadows of degenerated cells may be seen, with a number of small lymphoid cells apparently about to invade their territory.

In addition to these two extremes, the one of health, the other of extreme degeneration, altered cells are numerous, and indeed in a very advanced stage of pathological change. The cell and processes are enormously swollen, and the latter, many of which have apparently disappeared, are nearly completely free from chromatic substance. In the central portion of the cell the chromatic substance has been almost completely washed out, leaving a few flakes and pale, minute granules

scattered about in a faint, diffusely blue background. The periphery is bordered by a zone where these flocculent, pale granules are slightly more numerous [Plate III, Fig. 9]. The nucleus in the majority of these cells is partly protruding from the periphery, and encloses a very irregular, disintegrating nucleolus, surrounded by the finely granular blue deposit already mentioned. Outside the nucleus and along its cellular margin there is generally a fine layer of more deeply staining granules [Plate III, Fig. 9].

In certain of these cells, notably in those where the nucleus is not bulging from the periphery nor very eccentric, the fine deposit within the nucleus assumes a metachromatic, pale violet stain. By the concentration of these fine violet granules along the internal surface of the nuclear membrane, the nucleus is thrown into sharp relief against the pale blue of the more central portion of the cell. Some of these nuclei are large and only slightly irregular in outline, others small and greatly shrunken and distorted. As in the others the nucleoli are pale and undergoing the last stages of disintegration. In some of these latter cells, three zones of chromatolysis may be sharply distinguished, a very clear one immediately about the nucleus, a second not quite so clear separating it from the third or the border zone of larger masses at the periphery already referred to.

Rabbit VII, the last of this series, was first noticed to be paralyzed on the 29th day. Symptoms of recovery becoming marked, the animal was killed 64 days after the first inoculation.

In the cervical cord the ventral horn-cells do not differ in great degree from the normal type, although a number of slight variations exist. These consist for the most part in a slight increase in size of the cell, and a little irregularity in the shape and distribution of the Nissl bodies. This irregularity seems to consist, not in a breaking apart and destruction of the granules, but rather in the addition of new sharply staining granules to those already present, causing a little interference with the regular striped appearance of these bodies. This is usually accentuated about the nucleus, which is a little swollen and contains a few bluish granules, but is otherwise normal. Some cells are very deficient in the chromatic substance, especially about the

periphery. The chromatic granules about the nucleus in these cells are distinct and stain brilliantly with the methylene-blue.

At about the level of the second lumbar vertebra the cells are for the most part well formed and appear vigorous. The chromatic substance is dense and abundant, and characteristically arranged. The nuclei of these cells are of normal size and assume a very finely granular, almost homogeneous stain. Here and there are a few smaller cells with the chromatic substance distinct but somewhat finely divided.

On reaching approximately the level of the third lumbar vertebra a very different and striking picture is obtained. Corresponding doubtless to the paralyzed area are found large groups of cells which have a strong resemblance in their great size, eccentric nuclei and large clear dendrites to the cells in about this region in the previous experiment [Plate III, Fig. 9]. Here, however, the resemblance ceases. Instead of the pale, glassy, washed-out centre, the distorted, shrunken or swollen nucleus, and disintegrating nucleolus, the cell is filled with brightly staining sharp chromatic granules arranged in streaks shaped like the Nissl bodies, and traversing in a parallel direction the body of the cell toward the dendrites, or concentrically disposed about the nucleus, where the chromatic substance is especially abundant, being often especially dense along the inner edge of the nucleus [Plate III, Fig. 11]. The nucleus, instead of being greatly altered, is large, regularly spherical or oval, contains only a slight granular deposit and a normal deeply staining nucleolus. Many gradations in the amount of chromatic substance may be seen, but all these cells show this regular, apparently new formation of chromatic substance.

That these cells, as well as some of those described in the cervical region, are in a state of regeneration or restitution, is highly probable from their appearance alone, which corresponds also with that described as such by van Gehuchten (9), Marinesco (21) and others. In connection with this should also be taken into consideration the fact that this animal showed marked improvement, although the clinical symptoms and the alterations in the Nissl bodies are not always correlated.

A few cells seem to have lost their nuclei and to have completely degenerated. These are pale, shrunken forms contained in clear spaces, with a few blue flakes scattered within them, and at times invaded by wandering cells.

The part played by the nucleus in the stages of degeneration and of restitution is interesting. As is well known, if the nucleus be intact the cell may retain its power of regeneration. Should the nucleus, however, become completely degenerated or extruded from the cell, this power is lost (Nissl, 28). In many of the most altered cells, the nuclei which show marked signs of degeneration, as for example the metachromatic alteration in the granular deposit, are surrounded by a zone entirely free from chromatic substance, while in regenerating cells about the normal-looking nucleus, new granules are especially abundant [Plate III, Fig. 11]. In other words, as the nucleus gives out, the granules disappear from about it; as the nucleus recuperates, a new crop of granules appears along its cellular margin. This looks as if the nucleus controlled their formation, and thus, upon the supposition that they represent a nutritional element, presided in some way over the nutrition of the cell. The appearance of granules within the nucleus of altered cells may have something to do with this function, and may represent an exertion to replace the excessive destruction of the chromatic substance.

THE DORSAL ROOT GANGLIA IN EXPERIMENTAL CASES.

The dorsal root ganglia showed changes of a marked nature in the last four of these animals. In the ganglia from the lumbar region the altered cells are numerous. They are chiefly of two kinds and correspond in part to those already described in the human cases.

A few cells show a clumping of the chromatic granules into large swollen masses arranged in a row around the periphery and gathered about the centrally placed and only slightly altered nucleus. Between these two situations is a zone entirely free from chromatic substance [Plate IV, Fig. 16]. Others show no peripheral zone, but the chromatic substance is massed about the nucleus in the centre of the cell [Plate IV, Fig. 17].

The majority of the altered cells, however, show eccentric nuclei. In some few of these the chromatic substance is scattered at intervals in large flakes through the body of the cell [Plate IV, Fig. 15]; in others, the chromatic substance is finely divided, and in some of the latter the destruction seems to begin in one or more small areas [Plate IV, Fig. 13]. The nucleus is very eccentric, frequently bulging from the periphery, and encloses a pale, very irregular nucleolus. Altered cells, showing in particular eccentric nuclei, are most abundant in Rabbit IV, where most of the cells of the ganglia are thus affected.

In Rabbit V, on the contrary, there are few alterations, but there is great uniformity in the appearance of the cells, which are for the most part rich in chromatic substance, especially about their nuclei. This difference in the ganglia in these two animals corresponds very closely with the difference in the same animals between the conditions of the ventral horn cells.

In Rabbit IV, which died under the influence of an acute attack, the chromatic substance in both situations is much reduced; Rabbit V was on the road to recovery and an apparent restitution of chromatic substance is observed. This correspondence of external manifestations and internal alterations is not always so exact. The Nissl bodies are in a state of extremely unstable equilibrium and very easily influenced quantitatively. When such correspondence does exist, however, it is worthy of consideration.

The similarity of these alterations in the inoculated animals with those found in the human cases needs no further comment. The lesions are evidently uniform and constant. In both the human and the experimental series, the ventral horn cells show two forms of lesion. One is indefinite and characterized mainly by contraction or slight, irregular enlargement of the cells; by a chromatolysis marked in the periphery of the cell and at the entrance of the processes, with disintegrated granules more numerous about the nucleus which is either normal or together with the nucleolus is undergoing destruction in the centre of the cell. In some of these cells a process of vacuo-

lization is noted, and an apparent degeneration of the achromatic portion.

The other type of lesion is definite and well characterized by a regular enlargement of both cells and processes, a pale washed-out chromatolysis starting from the axone hillock and surrounding the somewhat altered nucleus, which is displaced to, and often protruding from, the opposite side. The achromatic portion is but little altered.

The first type of lesion is at no time predominant. It is most marked, however, in Rabbit III, and decreases somewhat in Rabbit IV. Both these animals were killed when the influence of the toxic substance on the whole system was at its height.

Without entering into a full discussion of the direct effect of toxic substances upon the nerve cell, attention may be called to the general resemblance between the alterations of this first type and those which have been produced by a number of investigators in different experimental intoxications, such as by arsenic, strychnine, alcohol, etc. These changes are doubtless due to the direct action of poisons on the cell. For a discussion of this subject reference may be made to the excellent articles of Marinesco (21), Lugaro (16), Flatau (6), and Goldscheider and Flatau (12). Although slight differences exist in the descriptions given by different authors, yet the lesions are essentially of the same type, and no doubt in our typhoid cases, too, they may be attributed to the direct action of the typhoid toxine.

Babes (1) has studied the changes in one case of typhoid inoculation in connection with a study of other infections, and describes only this first form of lesion, which he believes to be due to an actual invasion of the cell, as well as the tissues of the cord, by typhoid bacilli. Probably to this class belong especially the irregularly contracted and vacuolated cell forms with disintegrating nuclei.

Goldscheider and Flatau (10) and Moxter (25) have produced by experimental elevation of temperature, and Goldscheider with Flatau (11) and with Brasch (13), Déjerine (4), and Marinesco (20) have found in certain affections with high temperature in man, an alteration which corresponds closely with the pale blue non-vacuolated variety here described. Perhaps these forms may be referred to this

effect, though the temperature of the animals, when taken, was never particularly high.

Whether this lesion is due to direct toxic action, to high temperature, or to some other condition or to a combination of these need not be further discussed here. It is the more indefinite and not the predominant and characteristic lesion.

The second and characteristic lesion is that which predominates in all the human cases, and is especially advanced in the last of these. It is present to a greater or less degree in the whole animal series. It first shows itself in Rabbits I and II, to increase in III, to become particularly abundant in IV, when the other lesion begins to disappear. Its traces may be seen in the enlarged, apparently restituted cells described in Rabbit V, in which recovery from a half paralyzed condition was taking place. It is particularly advanced, though confined to the lumbar region, in Rabbit VI, which developed a paralysis of both hind legs. In Rabbit VII, paralyzed but recovering, it was manifested in the large cells, with eccentric nuclei, which were apparently undergoing regeneration.

This lesion, in its mode of onset, its general characteristics and progression, is similar in every way to the alterations produced in the corresponding cells in consequence of experimental section or other interference with the function of the axone, as described by Nissl (26, 27), Onufrowicz (31), Marinesco (21), Dutil (2), and many others. Specimens made in this way by Mr. Erlanger of the Johns Hopkins Medical School were kindly lent to me for purposes of comparison.

Moreover, an identical lesion has been discovered in human beings, where the axone has been injured or its function disturbed. Thus, Flatau (7) found this condition in two cases of amputation, and in one of thrombosis of the femoral artery; Adolf Meyer (24) in the facial nucleus, where the nerve had been compressed by inflammation in the internal auditory canal; and Barker (3) after injury to the fibres of the direct cerebellar tract in spinal meningitis found the same change in the cells of Clark's column.

Alterations in the dorsal root ganglia have also been studied after section of their peripheral nerves. Although this branch does not

correspond to the axone of the motor cell, yet it is apparently the only one which, when severed, produces alterations in the appropriate ganglion, as Lugaro (15) has shown by section of the cord above the ganglion examined, and by section of its peripheral nerve. By the latter procedure Lugaro observed alterations similar to those of cells with eccentric nuclei described in our typhoid cases, and shown in Plate III, Fig. 12, and Plate IV, Figs. 13, 14 and 15. R. A. Flemming (8) and Sadovsky (32) have described, in addition, cells with the chromatic substance massed about a centrally placed nucleus, as in Plate IV, Fig. 17.

From the character of the alteration in the motor cells and dorsal root ganglion, and its correspondence with the changes due to injury to the peripheral nerves, it seemed desirable to search outside of the nerve cells and cord for the primary lesion. This resemblance, coincident in both localities, cord and ganglia, at once suggested examination of the peripheral nerves. Unfortunately this was not done in the three human cases* nor in the first three rabbits or Rabbit VI.

THE PERIPHERAL NERVES IN EXPERIMENTAL CASES.

As the lumbar portion of the cord in all cases seemed chiefly involved the sciatic nerves were the ones excised, with some muscular connections, and in Rabbit V in connection with the cord.

Some parts of these were examined fresh, teased in glycerine, and stained with osmic acid. Other portions were hardened in Müller's fluid, and subsequently stained by Marchi's and Weigert's methods; and still others, hardened in Flemming's solution of formaline, were stained with carmine, safranin, polychrome methylene-blue, iron-hæmatoxylin and erythrosin. Some of the nerves hardened in formaline were stained by the method used by Kolosoff for heart muscle, which was found to give excellent results.

Degenerations were found in Rabbits Nos. IV, V and VII, the earliest case examined being Rabbit IV, which lived 52 hours after

*Since the completion of this paper I have examined portions of the sciatic nerve from a fatal case of human typhoid fever and have found definite parenchymatous degeneration similar to that described in the rabbit's nerves.

inoculation; numerous nerve fibres in the sciatic were found degenerated. These degenerations consisted in varicose swelling of the fibres, and breaking up of the neurokeratin and myeline sheath. In these vesicle-like spaces numerous small lymphoid cells and the shred-like remains of the structural network occur, while the axis cylinder is swollen, twisted, and no longer clear and homogeneous. About these areas the small-celled infiltration is quite noticeable.

In the later animals, especially in Rabbit VII, the altered myeline is broken up into balls and a deeply staining, granular detritus, which is collected for the most part into regular, oval or spherical bodies, suggesting the so-called "compound granular corpuscles" (Plate IV, Fig. 18). This suggestion is strengthened by the fact that, in sections hardened in formaline and Flemming's solution and stained with polychrome methylene-blue, safranin, etc., there may be seen lying within these swollen, degenerated areas, in addition to small lymphoid cells, large swollen cells with rather pale vesicular nuclei, placed somewhat eccentrically, in a granular-looking protoplasm. In such nerves the axis-cylinder is broken, twisted and much enlarged, the swollen club-like extremities projecting into the vesicular spaces. In Rabbit VII there is seen about the more degenerate spots a proliferation of the nuclei of the sheath of Schwann and of the interstitial tissue.

These lesions are especially marked in the gluteal, and some other lower branches of the sciatic nerve, and apparent especially in the intramuscular branches. The corresponding muscles are, in Rabbits V and VII, more or less affected, showing a fatty degeneration, staining black with osmic acid, loss of cross striation, atrophy of fibres, with, in the latter animal at least, some proliferation of the interstitial tissue.

Sections of the cord in Rabbits IV and VII were examined by Marchi's and the Pal-Weigert method, both in transverse and longitudinal section. A few nerve fibres, scattered indefinitely through the white substance, showed changes pointing to some slight degeneration of the myeline sheath but so slight in comparison with that of the peripheral nerves that they do not seem of much importance.

Marked degenerations are essentially peripheral and are such as are found in typical neuritis of the parenchymatous form, and call to mind especially those described by Sidney Martin (23) in his studies on diphtheria.

It would seem as if so profound an alteration of the nerves and muscle, a true Wallerian degeneration of the former, could scarcely be produced by primary lesions of the nerve cells, of the nature we have described. Such an interpretation of an alteration so superficial and slight as the disintegration and destruction of the Nissl bodies is known to be seems out of the question. It should be remembered that, except in a few cells, the achromatic substance was not much altered. Moreover, in one of the rabbits at least, the cells were quite restored to integrity, and in another undergoing a process of active restitution.

The changes could hardly have been primary in the muscles, as in Rabbit IV these were not much altered, while the nerves were quite extensively degenerated. Again, although ascending degeneration may take place in peripheral nerves, it is a process of longer duration. We must therefore conclude that we are dealing with a condition similar in its effects to section of a peripheral nerve, that is, with reaction on the nerve cell at a distance, brought about by changes in the nerves, the direct result of the action upon the latter of the typhoid poison.

Ballet (2) and Marinesco (17, 18, 19), in the cases of peripheral neuritis examined by them, found similar changes in the nerve cells, but are unwilling to infer definitely a primary alteration in the nerves, if much time has elapsed and the degeneration in the cells is so extensive that the achromatic portion is involved. This difficulty is obviated in the experimental lesions, where the whole development of the process may be traced, and which tend to show that some reliance is to be placed upon this form of lesion as an indication of the involvement of the peripheral nerves. Indeed, the peripheral nerves would not have been examined in this instance had not the lesion found proved so constant and characteristic, and in spite of the fact that it was expected that a direct toxic lesion would be discovered.

So far all experiments involving disturbance of the function of the axone produce constantly this one characteristic lesion. The fact that by infections, poisons, the use of high temperatures, the cutting off of the circulation from the cord, etc., this lesion is at times produced, along with other changes, proves nothing against its specificity, as all these injurious conditions might act as well directly on the peripheral nerves as on the cells. It is also held that in any morbid condition the disturbance of function of any one or all organs of the body produces abnormal conditions, which would mask the specific effect of the infectious agent. But it is well known that all organs or the same organs are not affected alike in the course of the same infection in different individuals, and hence such conditions and the lesions they might produce would not be constant. The lesions found in typhoid fever are constant. Therefore, where the lesion in question is predominant and constant it seems as if it should be referred to a peripheral effect of the infectious agent, until such effect can be proved to be excluded by examination of the peripheral nerves. The exclusion of these, however, is not an easy problem, as it has been shown by Nissl (26) that even such slight disturbance as contact of nerves with a grain of salt is sufficient to produce reaction upon the cells. Nissl also observed these cell alterations 6 hours after removal of part of the nerve, while Dutil (2) first notes degeneration in the proximal nerve stump by Marchi's method in 37 days. Thus an area of injury might be overlooked.

At least in these cases of typhoid infection it would seem that the toxine acts primarily upon the peripheral portion of the neurone, so far as this can be affected more in one portion than another, and there seems to be no reason why a unit which differs structurally in its parts should not differ as to these in its vulnerability. This means that the typhoid toxine is a powerful peripheral nerve poison, and that its effect is felt early and continuously, although it may be so slight as not to become manifest clinically, or be so slightly manifested as to be readily overlooked under existing means of observation in the height of the disease. Thus a slight loss of muscular control might well be veiled. At times, however, some complaint on the part of the

patient attracts the attention of the physician, and the condition is recognized. Such, for example, is probably the condition of "tender toes" not uncommon at this stage of the disease, as suggested by Dr. Osler in Vol. V of the Johns Hopkins Hospital Reports. Should the lesion be of a grave nature and produce a paralysis lasting into convalescence, then for the first time when the patient attempts to use these muscles, the true condition becomes apparent, and "develops" as one of the many varieties of post-typhoid neuritis. Among these occurs, though rarely, a diplegia, which is very resistant and slow to improve. By some this is, for clinical reasons, held to be a primary degeneration of the nerve cells, a true poliomyelitis. In two of the rabbits the same phenomenon developed but was due evidently to a peripheral nerve lesion, and it is not impossible that some of these nerves may fail to recover their peripheral connection, so that there ensues a complete degeneration of their appropriate cells (Flatau, 5; Marinesco, 18).

The aim of this investigation, the determination of the Nissl changes in typhoid infection, and, if found to be uniform, the assignment of some cause for this uniformity, is thus attained. I desire to express my thanks for valuable advice and assistance to Professors Welch, Flexner and Barker.*

Conclusions.—(1) The application of the Nissl method to the study of the motor cells of the spinal cord, and the nerve cells of the dorsal root ganglia in typhoid fever, shows that these cells regularly suffer pathological changes in the course of the infection.

(2) The alterations in the motor cells are more constant and of a severer grade than are those in the cells of the sensory ganglia. The more characteristic changes consist of disintegration, solution and destruction of the chromatic substance of the cell starting from the

* Since writing the above, a thesis by C. Voinot, delivered at Nancy, July 31, 1897, and entitled "Recherches anatomo-pathologiques sur la moelle épinière," fell into my hands. Of twelve cases of typhoid fever among other infections, five were examined by Nissl's method, and evidently with results much as above. The author, however, seems to lay stress upon alterations, perhaps definite, but slight, found in the cord and root fibres, the peripheral nerves not having been examined, and concludes that this disease belongs, with the others described, in the category of acute infectious myelitides, and that the alteration of the cells is coincident merely.

axone hillock and proceeding toward the nucleus. Coincidently the nuclei of the affected cells seek the periphery. Alterations are also suffered by the nucleus and nucleolus.

(3) While this central form of chromatolysis is the prevailing type of pathological change, disintegration, etc., of the Nissl bodies situated in the periphery of the cell and in the dendrites is also observed (peripheral chromatolysis).

(4) In experimental infection with typhoid bacilli in rabbits a similar series of lesions in the corresponding nerve cells in the spinal cord and ganglia is encountered.

(5) The main or central type of lesions discovered is identical with that found in man and animals after section, destruction, or even slight injury of the peripheral nerves.

(6) The examination of the peripheral nerves arising from the lumbar segment of the cord (the site in man and rabbit of the most profound changes) in rabbits inoculated with typhoid bacilli showed well-marked evidences of parenchymatous degeneration.

(7) It is probable that lesions of the peripheral nerves in typhoid fever in human beings are common and that the post-typhoid hyperæsthesias and paralyses are due to this cause.

(8) Restitution of the chromatic granules may take place in the affected nerve cells, the new formation beginning about the nucleus and extending through the protoplasm.

DESCRIPTION OF PLATES II–IV.

All figures were drawn with a Zeiss drawing apparatus; Figs. 3, 4 and 5 with Zeiss apochr. obj. 3 mm., oc. 4; the rest (except Fig. 18) with Zeiss apochr. oil immers. 2 mm., oc. 4.

[Measurements given are the longest and shortest diameters.]

PLATE II.

Fig. 1.—First human case: Cell from ventral horn, lumbar region, showing increase in size, characteristic form of chromatolysis, fine deposit in nucleus, vacuolated swollen nucleolus, and absence of chromatic substance in dendrites.

Size: cell, 78x36 μ; nucl. 24x18 μ; nucleol. 6x6 μ.

Fig. 2.—Similar to Fig. 1, showing also extension of process from axone hillock and slight dislocation of nucleus. Size: cell, 64x34 μ; nucl. 20x15 μ; nucleol. 7x7 μ.

Fig. 3.—Third human case; cell from ventral horn showing progression of the lesion shown in Figs. 1 and 2. Extreme eccentric position of nucleus with crumpled membrane and chromatic granules arranged along cellular margin. Nucleolus swollen but not much altered.

Size: cell, 63x33 μ; nucleol. 6x6 μ.

Fig. 4.—Similar to Fig. 3. Shows bulging of nucleus and extrusion (?) of nucleolus.

Size: cell, 75x15 μ.

Fig. 5.—Similar to Figs. 3 and 4. Somewhat more extensive chromatolysis, and swelling of cell.

Size: cell, 75x15 μ.

Fig. 6.—Rabbit III. Ventral horn cell. Indefinite "toxic" type of alteration; one small vacuole is shown containing a small oval body, resembling a bacillus.

Size: cell, 70x24 μ; nucleol. 4x4 μ.

PLATE III.

Fig. 7.—Rabbit II. Characteristic central lesion in earlier stage. Central chromatolysis beginning about axone hillock, and spreading toward nucleus, which is slightly eccentric. On the opposite side the Nissl bodies are swollen and disintegrating.

Size: cell, 49x32 μ; nucl. 18x12 μ; nucleol. 4x4 μ.

Fig. 8.—Rabbit IV. Same lesion further advanced. As yet not much eccentricity in nucleus. Compare with Figs. 1 and 2.

Fig. 9.—(Rabbit paralyzed.) Great enlargement of cell, and destruction of chromatic substance. Loss of processes. Eccentric nucleus. Disintegrating nucleolus. [Note double nucleoli not uncommon in rabbits.] (Compare with Fig. 4.)

Size: cell, 100x36 μ; nucl., 28x14 μ; nucleol., 5x4 μ.

Fig. 10.—Rabbit V. Type of large cells in ventral horn found above entrance of sciatic, and apparently nearly restored to integrity.

Size: cell, 90x60 μ; nucl. 26x20 μ; nucleol. 6x6 μ.

Fig. 11.—Rabbit VII. Type of cell of ventral horn in altered area apparently showing first stages of regeneration. (Compare with Figs. 8 and 9.)

Size: cell, 62x82 μ; nucl. 20x20 μ; nucleol. 5x5 μ.

Fig. 12.—Second human case; cell from lumbar dorsal root ganglion. Destruction of chromatic substance and eccentric nucleus.

Size: cell, 56x40 μ; nucleus, 16x10 μ; nucleolus, 5x6 μ.

PLATE IV.

Fig. 13.—Rabbit V. Similar to Fig. 14. Cell from lumbar dorsal root ganglion.

Fig. 14. Rabbit IV. Shows destruction of chromatic substance in two areas in cell body. Lumbar dorsal root ganglion.

Fig. 15.—Rabbit VII (paralyzed). Remains of chromatic substance scattered as large shreds through body of cell. Bulging of nucleus from periphery. Lower lumbar ganglion.

Fig. 16.—Rabbit VI (paralyzed). Clear zone separating peripheral collections of granules. Exaggeration of normal condition (?). Lumbar dorsal root ganglion.

Fig. 17.—Rabbit VII (paralyzed). Massing of chromatic substance about nucleus. Condition found in a number of cells in Rabbits V, VI and VII, but not always so well marked. Lowest lumbar root ganglion.

Fig. 18.—Rabbit VII. Sciatic nerve, hardened in Müller's fluid, stained by Marchi's method. Myeline sheath broken up, and detritus contained for the most part in granular cells. Zeiss objective D, ocular 4.

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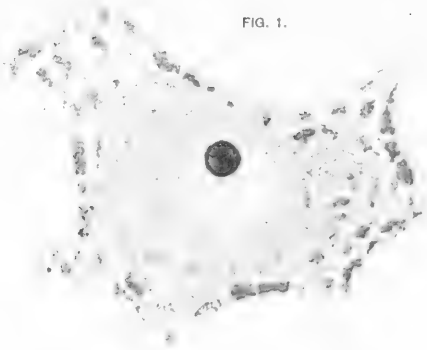
FIG. 1.



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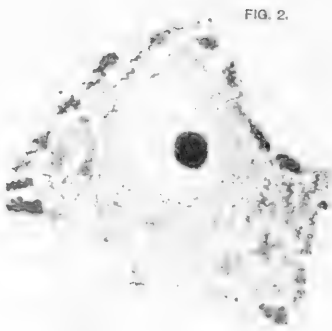

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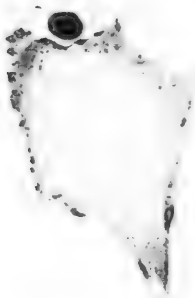

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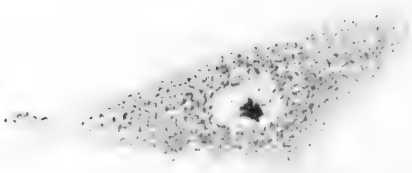

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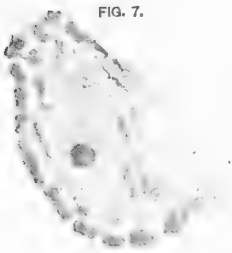

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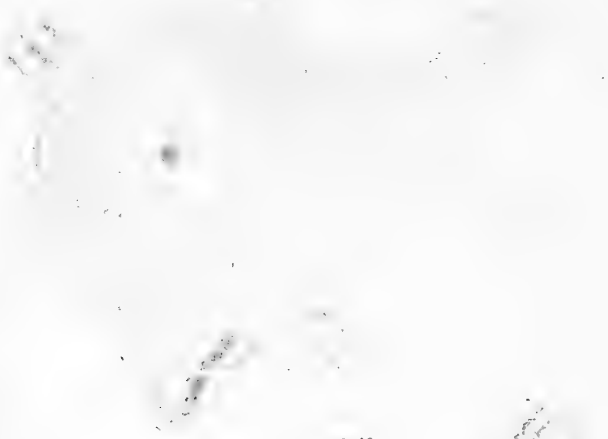

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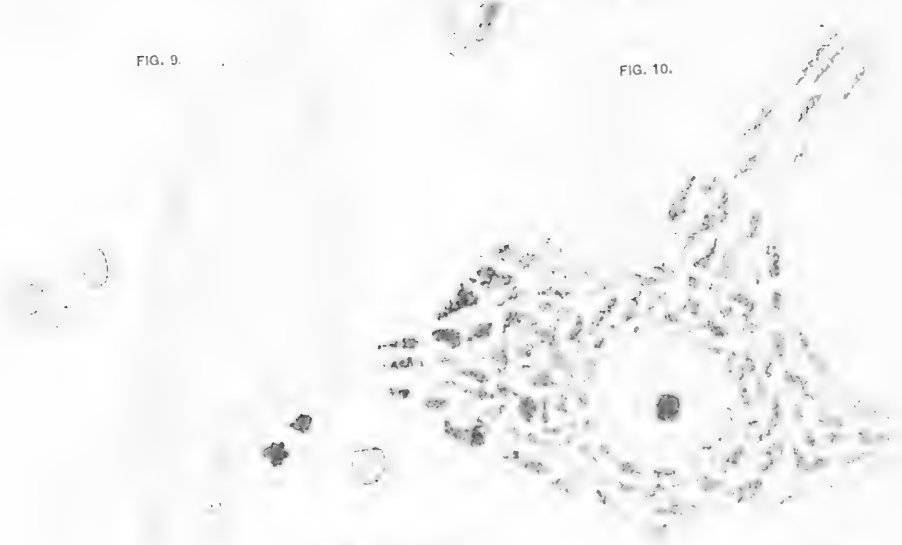

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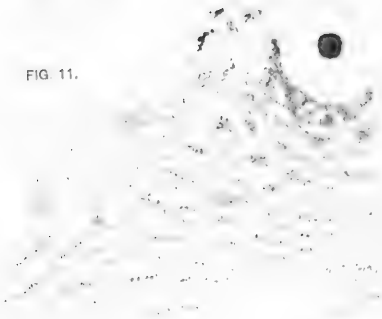

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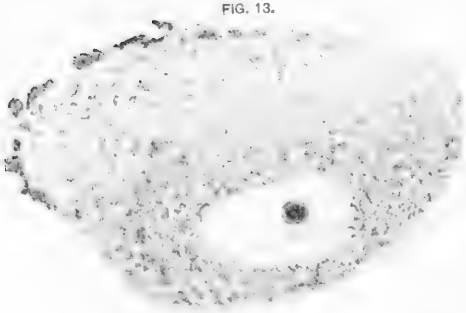

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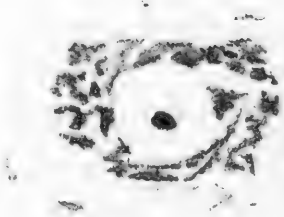

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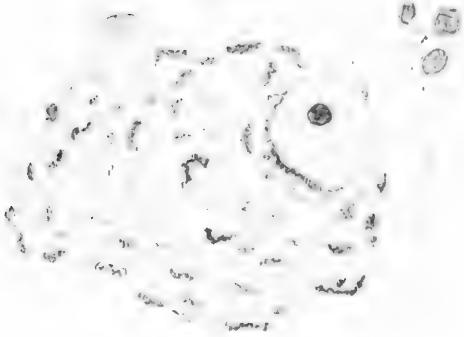

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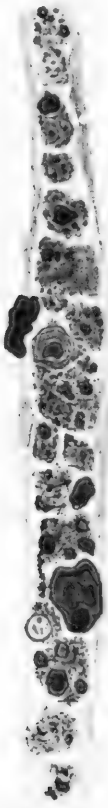

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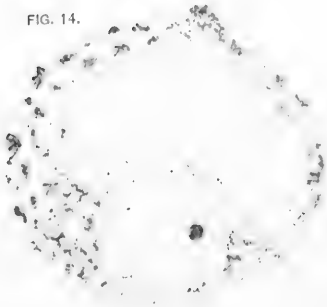

THE THERMAL DEATH-POINT OF TUBERCLE BACILLI IN MILK AND SOME OTHER FLUIDS.

By THEOBALD SMITH, M. D.

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In the comparative study of tubercle bacilli from various sources recently published,* an experiment was made to learn whether any differences towards heat would be manifested by cultures from man and from cattle. Pursuing the methods hitherto employed, I suspended the bacilli in milk and exposed the suspensions at 60° C. for different periods of time. The result was at variance with current notions of the resistance of tubercle bacilli at this temperature. A second trial gave a result quite at variance with the first, so that a third trial was made. Quite unintentionally I was drawn into a series of experiments extending over a period of more than 18 months, which have yielded several facts not anticipated, and destined to change somewhat our conceptions of the heat-resisting power of tubercle bacilli. Before detailing the tests, a brief review of the experiments of others will aid us in appreciating the necessity for the present work. In all my tests I restricted myself to the temperature of 60° C. in order to keep the work within certain narrow limits. I shall therefore confine my review to the determinations of others at that temperature.

Sternberg† in 1887 inoculated a guinea-pig with sputum exposed at 60° C. for 10 minutes. The animal remained unaffected.

Yersin‡ in 1888 tested an old glycerine bouillon culture in which the

* Theobald Smith: A Comparative Study of Bovine Tubercle Bacilli and of Human Bacilli from Sputum, JOURNAL OF EXPERIMENTAL MEDICINE, 1898, iii, 451.

† Sternberg, Disinfection and Disinfectants, etc., by the Committee on Disinfectants, appointed by The American Public Health Association, p. 148. Concord, N. H., 1888.

‡ Yersin, *Annales de l'Institut Pasteur*, 1888, ii, 60.

bacilli appeared "sporulating." Heated for 10 minutes at a temperature of 60° C. in small, sealed tubes, they still multiplied and produced disease in rabbits.

Grancher and Ledoux-Lebard* found avian cultures dead after an exposure of 20 minutes at 60° C., but not after 10 minutes. Human cultures were dead after an exposure of 15 minutes. Bonhoff† cultivated a human tubercle bacillus of unknown history in glycerinated calf's-lung bouillon. An exposure of such a culture for 20 minutes to 60° C. destroyed the bacilli.

de Man‡ in Forster's laboratory made a series of tests with tuberculous tissue. The thermal deathpoints established by him are now generally published in text-books. He used chiefly the disintegrated, semi-fluid, cheesy matter from tuberculous udders. His exposures in sealed tubes at 60° C. may be briefly summarized. After an exposure for 15, 30 and 45 minutes 2 guinea-pigs inoculated with material from each exposure became tuberculous. A repetition of this test yielded the same results. Two guinea-pigs, inoculated with material from two separate exposures for 60 minutes, remained well. Sputum exposed for 60 minutes was equally innocuous. From these results it has been generally assumed that it takes 60 minutes at 60° C. to kill tubercle bacilli.

Woodhead§ fed milk, from a tuberculous udder, which had been exposed for 15 minutes to 60° C. to 6 guinea-pigs without any effect. Similarly, milk heated for 30 minutes had no effect on 5 guinea-pigs fed with it. On the other hand, milk heated 15 minutes at 60° C. and injected into the peritoneal cavity produced tuberculosis in 2 of 3 guinea-pigs. Of three guinea-pigs inoculated in the same way with milk heated 30 minutes one became tuberculous. In another series of experiments with milk from a tuberculous udder no disease resulted from milk heated 25 or more minutes. Milk heated for shorter periods was not injected. Experiments were also made with milk to which ground and minced tuberculous tissue had been added. In such milk artificially infected, the tubercle bacilli were in some instances still alive after two hours' exposure.

This brief survey shows that these various tests were made under such different conditions that they cannot be utilized to define the thermal deathpoint of tubercle bacilli even if the results were fairly

* Grancher and Ledoux-Lebard, *Arch. de méd. expér.*, 1892, iv, 1.
† Bonhoff, *Hygienische Rundschau*, 1892, ii, 1009.
‡ de Man, *Arch. f. Hygiene*, 1893, xviii, 133.
§ Woodhead, Report of the Royal Commission on Tuberculosis, 1895, p. 146.

concordant. There was, therefore, ample justification for a repetition of these experiments with cultures of known history.

Milk was at first used exclusively as the suspending fluid for obvious reasons. Later on it became necessary to use simpler fluids, such as bouillon, distilled water and physiological salt solution to clear up the discordant results obtained with milk. The manipulations were modified from one test to another either to remove some supposed defect of former methods or to answer certain new queries which former tests had raised. The cultures used were all isolated by me and their history is given in a former publication.* Blood serum from the dog set at 75° to 76° C. was used exclusively as the culture medium. The suspensions were made by rubbing masses of bacilli against the inner surface of sterile test-tubes near the bottom until a fairly homogeneous coating had been formed. The rubbing was done with a heavy platinum wire beaten into the form of a slender spatula. The suspending fluid was then poured into the tube and thoroughly stirred. The resulting suspension still contained many clumps of bacilli made up of a few to 30 or more rods. The tubes thus prepared were exposed in a water-bath (Experiments I, II), or else other tubes were used to which definite quantities of the original suspension had been added. In all cases the suspensions were injected directly into the peritoneal cavity of guinea-pigs in order to give the bacilli the best opportunity for multiplication. Further details are given in the following account of the tests described in the order in which they were made.

Experiment I. June 4, 1897. Bovine culture II and sputum culture II were used. Growth of former a thin layer like ground glass in appearance; growth of latter much richer. Suspensions made, as described, in tubes of moderately heavy glass having an internal diameter of 15 to 18 mm. and provided with a glass cap ground to the tube and having a narrow ventilating tube above plugged with glass wool. This kind of tube differs from ordinary test-tubes simply in having more limited ventilation. About 10 cc. of milk, previously heated to 60° C., was added with a pipette and the suspension gently stirred. The tubes were then clamped in a water-bath kept at 60° C. with occasional fluc-

* Loc. cit.

tuations of 0.5° above or below this point. From each tube about 2 cc. of milk were removed with a sterile pipette at the end of 30, 45, 60 and 75 minutes. Each time the suspension was gently stirred by forcing it back from the pipette to counteract the settling of the bacilli. Great care was taken not to soil the walls of the tube above the level of the fluid. The tubes themselves were immersed to a depth of about 10 cm., the surface of the suspension being about 5 cm. below the surface of the water-bath.

The vitality of the bacilli was tested by injecting about 1 cc. into the peritoneal cavity of guinea-pigs. Further details are given in Table I.

TABLE I (EXP. I).—SUSPENSIONS IN MILK.

Designation of culture.	Time of exposure to 60° C.	Quantity injected in cc.	Weight of guinea-pigs in grammes.		Result.	Remarks.
			Initial.	Final.		
Bovine II	30 minutes.	1	548	565	—	Chloroformed in 18 days; 6 to 8 nodules on omentum.
	45 "	1	480	514	—	Chloroformed in 39 days; several nodules on omentum.
	60 "	1	435	415	—	Chloroformed in 26 days; several nodules on omentum.
	75 "	1	375	445	—	Chloroformed in 26 days; several nodules on omentum.
Sputum II	30 "	1	448	471	—	Chloroformed in 39 days; no lesions.
	45 "	1	412	474	—	Chloroformed in 43 days; 6 nodules on omentum.
	60 "	1	415	462	—	Chloroformed in 43 days; 10 nodules on omentum.
	75 "	1	375	350	—	Chloroformed in 26 days; 6 nodules on omentum.

In this first test no inoculations were made with unheated suspensions, as it was supposed that if it takes 60 minutes to kill tubercle bacilli at 60° C. the animals receiving the milk heated 30 minutes would be equivalent to control animals. This, however, failed to be the case, as all animals remained free from disease. This unexpected result suggested the possibility that the cultures may have been dead when used. An examination of the culture records showed, however, that subcultures from the cultures used in the experiment were fertile. There was nothing in the appearance of the cultures, only 11 days old, to suggest loss of vitality.

Experiment II. June 25, 1897. Sputum culture III and swine culture I used. Both 13 days old. Growth of sputum culture rich, that

of the other less so. The procedure differed from that of Experiment I in that the milk was added cold to the smeared tube. Due allowance for the warming of the fluid is made in the general summary given in Table IX (p. 230). Coverslip preparations showed the swine-culture suspension to be denser than the other. The inoculations are given in Table II.

TABLE II (EXP. II).—SUSPENSIONS IN MILK.

Designation of culture.	Time of exposure to 60° C.	Quantity injected in cc.	Weight of guinea-pigs in grammes.		Result.	Remarks.
			Initial.	Final.		
Sputum III	0 minutes.	1	555	260	+	Dying on 27th day; chloroformed. General tuberculosis.
	15 "	1	520	545	+	Chloroformed in 46 days; lesions in omentum, lymph glands, spleen, liver.
	30 "	1	475	493	—	Chloroformed in 47 days; about a dozen nodules on omentum.
	45 "	1	437	527	+	Chloroformed in 55 days; nodules and larger necrotic foci on omentum. Tuberculous retrogastric gland; minute yellow spots in liver. Thoracic glands enlarged but free from necroses.
Swine I	0 "	1	600	..	+	Dies in 13 days. Usual acute tuberculosis following abdominal injection.
	15 "	1	550	570	+	Chloroformed in 45 days. Tuberculosis of all thoracic and abdominal glands. A few foci in spleen and liver.
	30 "	1	482	492	—	Chloroformed in 47 days. Some nodules on omentum.
	45 "	1	422	425	+	Chloroformed in 55 days. General tuberculosis.

The outcome of this experiment was quite different from that of the preceding. Only the animals which received the milk suspension heated 30 minutes may be said to have received no living virulent bacilli. Those receiving milk heated 15 and 45 minutes from both cultures were distinctly tuberculous.

Experiment III. This may be disposed of briefly as it adds nothing new to the information gained by the other tests. It is, however, of

some general interest. July 1, 1897. The udder of a cow of which one-quarter was enormously enlarged by tuberculosis and the others atrophied and giving but little milk was the starting point. The tuberculous tissue was largely broken down into a caseous, diffluent mass. From one atrophied quarter a little milk was obtained with the hope that the thermal deathpoint of tubercle bacilli naturally suspended in that fluid might be obtained as a check upon the artificial suspensions. Though tubercle bacilli were not found in it with the microscope still the inoculations were made; 2 cc. of milk heated 0, 20, 35, 50 and 65 minutes were injected, each lot into 2 guinea-pigs. Killed from 6 to 9 weeks after inoculation, they were found free from disease. The milk was probably free from tubercle bacilli and the disease strictly limited to one-quarter.

Experiment IV. This test with bovine caseous material suspended in ordinary peptone bouillon was made mainly to determine whether the tubercle bacillus might exist in some more heat-resisting stage in caseous masses in which multiplication seems to be at a standstill.

TABLE III (EXP. IV).—BOUILLON SUSPENSIONS OF CASEOUS MATTER (CATTLE).

Time of exposure to 60° C.	Quantity injected in cc.	Weight of guinea-pigs in grammes.		Result.	Remarks.
		Initial.	Final.		
0 minutes.	0.6	342	404	+	Chloroformed in 42 days. General tuberculosis.
0 "	2.	482	484	+	Chloroformed in 27 days. General tuberculosis.
0 "	2.	657	640	+	Chloroformed in 39 days. General tuberculosis.
20 "	"	646	693	—	Chloroformed in 31 days. One nodule on omentum.
" "	"	383	505*	—	Chloroformed in 55 days. No lesions.
35 "	"	445	622	—	Chloroformed in 54 days. No lesions.
" "	"	612	701	—	Chloroformed in 31 days. No lesions.
50 "	"	485	593†	—	Chloroformed in 106 days. No lesions.
" "	"	339	445	—	Chloroformed in 31 days. One nodule on omentum.
65 "	"	567	592†	—	Chloroformed in 129 days. One nodule on omentum.
" "	"	329	418	—	Chloroformed in 18 days. No lesions.

* Pregnant when chloroformed.

† Had given birth to a litter of healthy young 4–6 weeks before it was chloroformed.

Nov. 26, 1897. Completely caseous matter from the lungs of a cow was scraped into a small dish and kept on ice until next day. It was

ground in a sterile mortar with bouillon and the turbid fluid passed through several thicknesses of linen. In the densely turbid, pale yellowish fluid, consisting microscopically of fatty debris chiefly, tubercle bacilli were not found. 7 cc. were carefully transferred with a pipette to a number of test-tubes with ground glass cap. These were immersed in the 60° bath so that the surface of the fluid was 5 cm. below the level of the bath. A tube was removed at the end of 20, 35, 50 and 65 minutes. Table III (p. 222) shows that even 20 minutes was sufficient to destroy the bacilli, and that in caseous tissue tubercle bacilli do not possess any greater power of resistance to heat than in cultures on serum.

TABLE IV (EXP. V).—A. Suspension of Bovine Bacilli in Bouillon.

Time of exposure to 60° C.	Quantity injected in cc.	Weight of guinea-pigs in grammes.		Result.	Remarks.
		Initial.	Final.		
0 minutes.	0.5	581	468	+	Dies in 17 days. Acute tuberculosis.
20 "	1.5	544	582	—	Chloroformed in 52 days; a dozen nodules on omentum.
(Bacilli from nodules of preceding case.)		340	405	—	Chloroformed in 37 days; no lesions.
35 minutes.	1.5	554	638	—	Chloroformed in 43 days; a dozen nodules on omentum, slight hyperplasia of lymph glands.
50 "	"	528	607	—	Chloroformed in 49 days; six nodules on omentum.
65 "	"	349	498	—	Chloroformed in 58 days; nodules on omentum, one in ovarian ligament.
B. Suspensions in Milk.					
(Milk not infected.)	3.5	421	..	—	Dies in 7 days of streptococcus infection; abscesses in omentum and between spleen and liver.
(Milk not infected.)	"	401	..	—	Dies in 7 days of streptococcus infection.
0 minutes.	1.5	591	430	+	Dies in 15 days; acute tuberculosis.
20 "	"	401	478	+	Chloroformed in 52 days. General tuberculosis.
35 "	"	510	671	+	Chloroformed in 43 days. Tuberculosis of lymph glands; slight infection of liver and spleen.
50 "	"	401	514	+	Chloroformed in 49 days. General tuberculosis.
65 "	"	369	510	+	Chloroformed in 58 days. General tuberculosis.

Experiment V. The preceding tests taken together suggested that tubercle bacilli may be much more resistant to heat in milk than in

bouillon. This suggestion was tested in the following manner with bovine culture III:

A. Bouillon series, Feb. 15, 1898. The culture, 17 days old, was suspended in bouillon having a reaction equivalent to 1.45 per cent. of a normal acid solution (phenolphtalein). Ordinary, cotton-plugged thin-walled test-tubes were used, 15 to 18 mm. in diameter and 15 cm. long. Each tube received 2 cc. of the suspension and was immersed to a depth of 10 cm. in the bath. One was removed at the end of 20, 35, 50 and 65 minutes.

B. Milk series. The tubes were prepared in the same way. The milk was about 6 hours old, kept in the refrigerator. Acidity 1.9 per cent.

The result of the inoculations given in Table IV (p. 223) shows that the bacilli in the bouillon suspensions had been killed, while some of those in the various milk tubes had survived.

TABLE V (EXP. VI).—A. SUSPENSION OF BOVINE BACILLI IN WATER.

Time of exposure at 60° C.	Quantity injected in cc.	Weight of guinea-pigs in grammes.		Result.	Remarks.
		Initial.	Final.		
15 minutes.	1.5	304	548	—	Killed in 64 days. Some nodules on omentum.
25 "	"	358	416	—	Killed in 35 days. Some nodules on omentum.
		B. SUSPENSION IN MILK.			
(Milk not infected.)	3.5	732	..		Dies in 6 days. Abscesses in abdominal cavity. Streptococcus infection.
(Milk not infected.)	"	379	350	—	Chloroformed in 31 days. Large abscess in abdominal cavity due to minute bacilli.
(Milk not infected.)	"	444	591	—	Chloroformed in 59 days. No lesions. Milk from same dairy but from a lot 11 days later than preceding.
0 minutes.	1.	342	..	+	Dies in 9 days. Acute tuberculosis and streptococcus infection.
20 "	1.4	317	360	—	Killed in 61 days. Several nodules on omentum.
35 "	2.	409	411	+	Killed in 29 days. Tuberculosis of omentum, peritoneum and lymph glands.
50 "	1.5	363	453	—	Killed in 64 days. Several nodules on omentum.
65 "	2	370	508	+	Killed in 64 days. Slight tuberculosis of lymph glands of abdomen, liver and spleen.
80 "	2	420	595	—	Killed in 64 days. A few nodules on omentum, 2 minute ones on testicles.

Experiment VI. April 28, 1898. This is an exact counterpart of Experiment V with certain minor modifications. The culture is a young (seven-day) culture of bovine bacillus IV. The suspensions were made, as before, in milk, and in distilled water. They were exposed in cotton-plugged test-tubes. The ready destruction of the bacilli in water, their irregularly persistent vitality in the milk is again demonstrated in Table V (p. 224). Cultures from the water suspensions confirmed the inoculation results. The control unheated suspension gave rise to a vigorous culture on dog's serum, the cultures from the heated suspensions remained sterile.*

TABLE VI (EXP. VII).—A. Suspension of Bovine Bacilli in Water.

Time of exposure to 60° C.	Quantity injected in cc.	Weight of guinea-pigs in grammes.		Result.	Remarks.
		Initial.	Final.		
0 minutes.	..	..	..	+	Culture positive.
12 "	1.5	406	474	—	Culture negative. Animal killed in 75 days. Slight hyperplasia of mesenteric and sternal glands. Several nodules on omentum and one on abdominal wall.
22 "	1.5	366	490	—	Culture negative. Animal killed in 86 days. Some nodules on omentum.
B. Suspension in Milk.					
(Milk not infected.)	2.0	384	664	—	Chloroformed in 75 days. No lesions.
0 minutes.	0.5	375	270	+	Dies in 32 days. General tuberculosis. Abscess in omentum containing cocci.
17 "	1.5	367	448	—	Killed in 56 days. Some nodules on omentum.
32 "	"	353	443	—	Killed in 56 days. Some nodules on omentum.
47 "	"	403	451	—	Killed in 56 days. Some nodules on omentum.
62 "	"	385	508	—	Killed in 56 days. Some nodules on omentum, and cheesy focus in subcutis at point of injection.
77 "	"	395	498	—	Killed in 56 days. Some nodules on omentum.

Experiment VII. May 18, 1898. The procedure differed from that of former tests in that the suspensions were exposed in com-

* Cultures from milk suspensions were not attempted because of the anticipated presence of contaminating bacteria. To sterilize the milk before use by discontinuous boiling would obviously fail to imitate natural conditions.

pletely submerged, sealed glass tubes. An old culture was used to determine if more resistant bacilli appear after a time.

Bovine IV, grown for 30 days on the same lot of dog's serum on which the young culture of the preceding experiment had grown was used. The milk had an acidity of 1.7 per cent. Parallel exposures in distilled water were also made. The tubes were made of ordinary glass tubing having an internal diameter of 6 mm. Both ends were drawn out in the flame. One end was sealed at once. About 2 cc. of the suspension was inserted through the other opening with a delicate pipette and the latter then sealed in the flame. This proved a better method than drawing the suspension up first and then sealing as it prevented the usual sputtering when the sealing is attempted and the indiscriminate distribution of tubercle bacilli. Each sealed tube contained one-half to one-third its volume of air. They were laid horizontally on a rack about 7 cm. below the surface of the bath close to the thermometer bulb. The results of the inoculation are given in Table VI (p. 225).

The outcome of this test was a surprise, for not one of the guinea-pigs inoculated with heated suspensions became tuberculous. For the water suspensions the culture test agreed with the animal test. A vigorous culture was obtained from the unheated suspension in two weeks. The other tubes were sterile after 4 weeks. It will be at once thought that the use of the sealed tubes accounts for this, but why should the suspensions in fluids other than milk give uniform results in test-tubes? The remaining two experiments, I think, give a satisfactory answer to this query.

Experiment VIII. August 12, 1898. In this test bovine culture VI was used. The bacilli were exposed in sealed tubes. The milk, kept over night in the refrigerator, had a heavy layer of cream on it next morning. It was thoroughly mixed before use. Acidity 1.5 per cent. Suspensions in normal salt solution were also made. The result of the test given below in Table VII did not differ materially from that of the preceding test. In the 12-minute tube the bacilli suspended in salt solution were not all destroyed. The guinea-pig inoculated with the milk suspension exposed for 17 minutes had a suspicious subcutaneous lymph gland with which unfortunately fresh inoculations were not made, so that this case remains in doubt. However, the lesion was so slight, if due to living bacilli, that one bacillus may have caused it.

TABLE VII (EXP. VIII).

A. Suspension of Bovine Bacilli in Normal Salt Solution.

Time of exposure to 60° C.	Quantity injected in cc.	Weight of guinea-pigs in grammes.		Result.	Remarks.
		Initial.	Final.		
12 minutes.	1.5	338	464	+	Chloroformed in 106 days. Tuberculosis of both testicles, of left popliteal, and of lumbar gland. All foci softened. Bronchial glands tuberculous; still firm.
22 "	"	376	508	—	Chloroformed in 106 days. Two small, softened nodules on omentum; one on testicle.

B. Suspension in Milk.

(Milk not infected.)	3.5	251	640	—	After 105 days weighs 640. Alive after 5½ months.
0 minutes.	0.5	373	..	+	Dies in 9 days of acute tuberculosis.
17 "	1.5	388	494	+	Killed in 57 days. Slightly enlarged and partly necrotic popliteal gland.
32 "	"	398	532	—	Killed in 57 days. Several 2–3 mm. nodules on omentum.
47 "	"	366	545	—	Killed in 63 days. No lesions.
62 "	"	298	423	—	Killed in 63 days. Several nodules on omentum.
77 "	"	267	479	—	Killed in 63 days. Slightly conspicuous retrogastric and thoracic lymph glands.

Experiment IX. Nov. 17, 1898. In this test bovine culture III, used in Experiment V, was again employed. The suspensions were prepared from a vigorous culture 13 days old. Its vitality was demonstrated by the rapid, rich growth of a subculture made at the same time with the suspensions. The bacilli suspended in distilled water were exposed in the test-tubes with ground glass cap, each tube receiving 2 cc. The milk suspensions were exposed both in these tubes (4 cc.) and in sealed tubes. The outcome of the tests made thus far had suggested the theory that the occasional survival of bacilli leading to the irregular results tabulated might be due to the formation of the surface pellicle into which the bacilli are carried by particles of fat. Here their thermal deathpoint might be higher than when submerged in a watery fluid. Hence the pellicle which had formed during the exposure in the test-tubes was in part removed with a platinum loop and stirred in with the rest of the milk to be injected. The milk used had a layer of cream (formed over night) equivalent to 14 per cent. of the whole column. Acidity 1.67 per cent. The bore of the sealed tubes was 4 mm. They contained about 1.5 cc. milk and some air. Coverslip preparations dem-

onstrated the presence of large numbers of bacilli chiefly in clumps. The results are summarized in Table VIII.

TABLE VIII (EXP. IX).—A. Suspension of Bovine Bacilli in Distilled Water Exposed in Culture Tubes with Glass Cap.

Time of exposure to 60° C.	Quantity injected in cc.	Weight of guinea-pigs in grammes.		Result.	Remarks.
		Initial.	Final.		
0 minutes.	0.5	384	..	+	Dies in 25 days of tuberculosis. Suspension made from the same culture 12 days after the general experiment to replace milk controls (see below).
10 "	1.5	332	418	+	Killed in 62 days. Tuberculosis of lymph nodes, liver, spleen, peritoneum.
15 "	1.5	372	438	+	Killed in 61 days. Tuberculosis of lymph nodes chiefly.
20 "	1.5	302	331	+	Killed in 61 days. Generalized tuberculosis.
B. Suspensions in Milk in Sealed Pipettes.					
(Milk not infected.)	4.0	524	..		Dies in 48 hours of streptococcus peritonitis and septicæmia.
0 minutes (control).	0.5	537	..	+	Dies in 6 days of streptococcus infection and tuberculosis (?).
7 minutes.	1.5	450	500	+	Killed in 62 days. Tuberculosis of lymph nodes. Slight infection of liver and spleen.
12 "	1.5	441	503	+	Killed in 62 days. Slight disease of lymph nodes. Tuberculosis of left serotal sac.
17 "	0.5	491	605	—	Killed in 62 days. Slight localized thickening of omentum.
32 "	1.5	354	..	—	Dies in 15 days.* Six or seven small nodules on omentum; 2 on mesocolon.
47 "	1.5	368	492	—	Killed in 62 days. Several softened nodules on omentum.
62 "	1.5	303	330	—	Killed in 62 days. Some local thickenings of omentum.
C. Suspensions in Milk in Culture Tubes as in A.					
20 minutes.	1.5	378	275	+	Dies in 38 days. General tuberculosis.
35 "	1.5	272	291†	+	Dies in 52 days. General tuberculosis.

* Diphtheritic paralysis. Had been used for antitoxin test 24 days before inoculation. Only a slight transient swelling followed.

† Weight 10 days before death.

This test strengthens the supposition that the pellicle which forms on milk in test-tubes during heating is responsible for the increased resistance of milk suspensions of tubercle bacilli and the irregular

results hitherto obtained.* The two guinea-pigs inoculated with milk exposed 15† and 30† minutes together with some of the pellicle died of tuberculosis in 38 and 52 days respectively, while two other guinea-pigs inoculated with suspensions in milk and water exposed only 5† minutes were found with but slight lesions after 60 days.

A summary of all the tests made is given in Table IX (p. 230), together with some additional data concerning the cultures used. The figures in parentheses following the figures giving the total exposure at 60° C. are an approximate correction representing the actual time of exposure at that temperature.

A brief explanation of the tabulated autopsy notes of the guinea-pigs seems necessary in view of the fact that the conclusions to be drawn rest chiefly on them. It will be observed from the tables that in many cases small nodules were found on the omentum without any traces of tuberculosis in other organs, even the nearest lymph nodes. These nodules were studied histologically at different stages, although it was manifest from the purely local character of the lesions and the knowledge already gained from the researches of Prudden and Hodenpyl and of Straus concerning the action of dead tubercle bacilli that these nodules were the product of dead bacilli swept together into a mass in some way on different places on the omentum. It would be beyond the scope of this article to go into minute details concerning the structure of these nodules. Suffice it to say that within two to four weeks after the intra-peritoneal injection they are one to three mm. in diameter and translucent in appearance. They consist, then, of epithelioid and lymphoid cells, among which are many nuclear figures. In this stage the mass may contain roundish cell groups closely resembling the "germinative centres" of follicles in lymph nodes. In later stages the larger nodules undergo softening centrally, and the creamy-white mass there formed contains readily

* This pellicle, which is familiar to all who have scalded milk, is a feebly cohesive mass easily washed out in patches for microscopic examination by diluting the heated milk in water. The patches consist of fat globules and an amorphous, cohesive substance of slight refrangibility, by which they are evidently held together.

† These figures are corrected by subtracting the time required to bring the fluid to 59.5° C.

TABLE IX.—Summary of Experiments.

Source of bacilli.	Total period of cultivation on dog's serum.	Age of culture used.	Nature of suspending fluid.	Tubes used.	Result. (The figures signify minutes, the sign + tuberculosis, the sign — no tuberculosis.)
Caseous material from cow's lung.	.....	....	Peptone bouillon	Culture tubes with glass cap	—20(15), —35(30), —50(45), —65(60)
Bovine culture III	9 mos. 27 days	17 days	Peptone bouillon	Test-tubes with cotton plug	—20(15), —35(30), —50(45), —65(60)
Bovine culture IV	11 mos. 26 days	7 days	Distilled water	Test-tubes with cotton plug	—15(10), —25(20)
Bovine culture IV	13 mos.	30 days	Distilled water	Sealed glass tubes	—12(10), —22(20)
Bovine culture VI	7 mos. 19 days	10 days	Normal salt sol.	Sealed glass tubes	+12(10), —22(20)
Bovine culture III	18 mos. 29 days	13 days	Distilled water	Culture tubes with glass cap	+10(5), +15(10), +20(15)
Bovine culture II	6 mos. 7 days	11 days	Milk	Culture tubes with glass cap.	—30, —45, —60, —75
Sputum culture II	6 mos. 14 days	11 days	Milk	Culture tubes with glass cap	—30, —45, —60, —75
Sputum culture III	4 mos. 10 days	13 days	Milk	Culture tubes with glass cap	+15(10), —30(25), +45(40)
Swine culture I	13 mos. 17 days	13 days	Milk	Culture tubes with glass cap	+15(10), —30(25), +45(40)
Bovine culture III	9 mos. 27 days	17 days	Milk	Test-tubes with cotton plug	+20(15), +35(30), +50(45), +65(60)
Bovine culture IV	11 mos. 26 days	7 days	Milk	Test-tubes with cotton plug	—20(15), +35(30), —50(45), +65(60)
Bovine culture IV	13 mos. 26 days	30 days	Milk	Sealed glass tubes	—17(15), —32(30), —47(45), —62(60), —77(75)
Bovine culture VI	7 mos. 19 days	10 days	Milk	Sealed glass tubes	+17(15), —32(30), —47(45), —62(60), —77(75)
Bovine culture III	18 mos. 29 days	13 days	Milk	Sealed glass tubes	+(75), +12(10), —17(15), —32(30), —47(45), —62(60)
Bovine culture III	18 mos. 29 days	13 days	Milk	Culture tubes with glass cap	+20(15), +35(30)

demonstrable tubercle bacilli. The softening is essentially a process of caseation, never of suppuration. The necrosis is sharply demarcated from the enveloping dense outer layer of the nodule and thereby differs clearly from the progressive peripheral invasion of living bacilli. It is not possible, however, to make trenchant distinctions between early lesions due to living and to dead bacilli, and so far the progressive metastatic nature of the disease as recognized with the unaided eye seems to me the most satisfactory guide, provided a sufficient period of time (at least 50 days) be allowed to elapse between the inoculation and the examination of the animal. In but one instance (Experiment V) the bacilli from a softened focus due to dead bacilli were injected into a guinea-pig. The result was negative.

There is nothing of importance to be recorded concerning the macroscopic characters of the inoculation disease in guinea-pigs. Its protean character, as exemplified by the diverse localizations, makes a very thorough examination of every animal essential. In general, the retarded disease has its chief seat in the lymph nodes, which may attain a large size. This is particularly true of the retrogastric and lumbar, and of the bronchial and neighboring sternal nodes, whose large dimensions with visibly intact lungs suggest the bovine type of disease. In several cases the testicles and surrounding scrotal tissue were involved precisely as in glanders (following either subcutaneous or intro-abdominal inoculation). It is probable that in these experiments male guinea-pigs would be preferable, since the disease may be recognizable during life. In no case was there any evidence of air infection. In fact, I have not encountered it at any time among the animals kept for several years in the room used for these experimental animals. It is again evident from these tables that the weight of the guinea-pig is no guide in determining the presence or absence of tubercular foci.

The foregoing experiments demonstrate that tubercle bacilli are no more resistant to heat than many other bacilli not producing spores, and that at 60° C. destruction is complete in 15 to 20 minutes. Even after exposures lasting 10 minutes the bacilli were dead in most

instances. After 5-minute exposures the inoculation disease produced in guinea-pigs was greatly retarded, even though three times the control dose was injected.* When, however, milk is used as the suspending fluid, the formation of a surface pellicle into which bacilli are carried by fat globules shields them from the effect of the heat so that they may survive an exposure of 65 minutes. The peculiarly irregular results obtained by the Royal Commission† are probably to be explained in the same way. The importance of a clear understanding of this phenomenon in the pasteurization of milk is obvious, and it remains to be seen how far bottled milk may be freed from tubercle bacilli without resorting to the higher temperature of 68° C. now generally employed. Probably a complete immersion, or else a complete filling of the receptacle, may furnish the conditions desired.

These conclusions may be objected to on the ground that the tubercle bacilli may have suffered in power of resistance during cultivation, but the experiment with caseous material from cattle (IV) will, I think, silence this objection. The different results obtained by de Man, already quoted and now generally accepted, are probably due to the fact that he used caseous material or else tuberculous tissue, which he ground up in a mortar and only diluted with salt solution "when necessary." Such dense suspensions cannot be used to determine the thermal deathpoint to be compared with those of other bacilli, nor can they be regarded as imitating the conditions under which tubercle bacilli appear in milk.

The method of using narrow sealed tubes has shown itself superior to that which employs ordinary test-tubes so far as milk suspensions are concerned. When other fluids were used the results were unequivocal and in harmony with the other method, excepting, per-

* It should be borne in mind that the suspensions used were quite dense. In a small drop spread out on cover glasses, dried and stained, large numbers of bacilli singly and in clumps were seen in every field of a $\frac{1}{12}$ objective.

† Woodhead, loc. cit.

haps, in the last experiment, in which an increased resistance of the tubercle bacilli in water is manifest.*

It simply remains to point out the possibility that the resistance of other pathogenic bacteria to heat may be increased in milk. Hitherto all practical details in sterilization have been based on the thermal deathpoint of bacteria in bouillon and other fluids.

Conclusions.

1. Tubercle bacilli when suspended in distilled water, normal salt solution, bouillon and milk, are destroyed at 60° C. in 15 to 20 minutes. The larger number are destroyed in 5 to 10 minutes.

2. When tubercle bacilli are suspended in milk, the pellicle which forms during the exposure at 60° C. may contain living bacilli after 60 minutes.

* This test was repeated, after the completion of this article, for another purpose with the same culture and under the same conditions, and the same resistance to a net exposure of 15 minutes was noted. In the suspensions in water delicate floating fragments of a pellicle of bacilli were noticed which could not be submerged by vigorous shaking. Pellicles behaving similarly are frequently seen on bouillon cultures of colon and typhoid bacilli. That this phenomenon was related to the slightly increased resistance of the culture seems probable in view of the results obtained with milk suspensions in sealed pipettes. In any case those who are inclined to repeat these tests would do well to immerse completely all suspensions whether in milk or water so as to expose such floating bacilli to the same conditions of heat and moisture under which the suspended bacilli are.

THE CHANGES PRODUCED BY THE GROWTH OF BACTERIA IN THE MOLECULAR CONCENTRATION AND ELECTRICAL CONDUCTIVITY OF CULTURE MEDIA.*

By G. N. STEWART, Cleveland.

PLATES V–VIII.

By determining from time to time the freezing point of a liquid in which bacteria are growing it is possible to estimate the changes that take place in the molecular concentration.†

By determining the electrical conductivity a measure of the number of dissociated ions in unit volume of the solution may be obtained. The amount by which the freezing point is depressed below the freezing point of distilled water is directly proportional to the molecular concentration when the dissolved substances are incapable of hydrolytic dissociation, like cane sugar or proteids for instance. In the case of dissociable substances, like sodium chloride, the same is true when we reckon each ion as a molecule. The relation between the electrical conductivity and the concentration of the electrolytes is not so simple, since the specific conductivity of a solution depends not only on the number of dissociated molecules in a given volume, but also on the velocities of the ions. But there is always a relation, and when this can be established for a particular case, it can be used to calculate the concentration. For example, when only one electrolyte is present, the concentration can be at once determined from the electrical conductivity by interpolating values in a table showing the relation between the conductivity of the substance and the amount of it in solution. If more than one electrolyte be present, the electrolytes may be such as have approximately equal ionic veloc-

* This paper was read before the British Medical Association (Section of Physiology) at Edinburgh, July, 1898.

† The molecular concentration may be defined as the number of gramme-molecules of the dissolved substance in a litre of the solution.

ities. In this case the electrical conductivity can be taken as directly proportional to the total concentration of the electrolytes. If more than one electrolyte is present and the ionic velocities are widely different, then a quantitative determination of one or more of the electrolytes may be made by chemical methods, and the concentration of those that remain be deduced from the electrical conductivity. And even in complex solutions, when without actually knowing the ionic velocities we know the limits between which they must lie, we can often determine from the electrical conductivity whether the number of dissociated molecules in a given volume is increasing or diminishing, and, within certain limits of accuracy, by how much it is increasing or diminishing. This calculation can be controlled and the limits of error narrowed by taking account of the total molecular concentration (including that of the dissociated ions) deduced from observations on the freezing point. For instance, if we found that certain bacteria growing in solution of albumin caused a diminution in the freezing point without any change in the electrical conductivity, this would indicate that the large albumin molecules were being broken down into smaller, but as yet non-dissociable, molecules, possibly into peptones. If, later on, we found that while the molecular concentration, as indicated by the lowering of the freezing point, was still increasing, the electrical conductivity also began to increase, we should have to conclude that in the course of the decomposition the point had now been reached where some of the comparatively small molecules were at length dissociable. If the molecular concentration and the electrical conductivity were found both to increase from the beginning of the bacterial growth, it would be proved that the decomposition even at the beginning went as far as the production of the dissociable electrolytic molecules.

With the view of obtaining some preliminary notion of the magnitude of the changes which were to be expected, I made a series of experiments on the growth of ordinary putrefactive bacteria in blood and serum, and of pure cultures of certain bacteria in solutions of Witte's "peptone." The freezing-point determinations were made by means of a modification of Beckmann's apparatus, and the electrical resistance was measured by the telephone method of Kohlrausch. It

will be seen from the subjoined specimens of the results that very striking changes are observed both in the total molecular concentration and in the electrical conductivity, which, upon the whole, run a parallel course. Thus, in Experiment I, dog's defibrinated blood and serum were allowed to putrefy for 6 weeks in a bath at a temperature of 37° to 39° C. The electrical conductivity of the blood in that time was increased more than eleven-fold, and the freezing point was depressed in proportion. There was a similar change in the serum, although the maximum was much less. Curves plotted from the results of this experiment are shown in Plate V.

In Experiment II, three specimens of blood taken at the same time from the carotid artery of a dog were allowed to putrefy first at the air temperature, then at 35° C., and then at 40° to 43° C. Of course in all the experiments evaporation was prevented. The course of the decomposition is shown in the curves of Plate VI, which represent the electrical conductivity. It is abruptly hastened by raising the temperature to 35° C. Ultimately the conductivity reaches a maximum and then remains practically constant for an indefinite period. At the time when the maximum was reached the blood was seen to be crowded with bacteria which had formed spores. It is to be presumed that at this point the conditions had become unfavorable for the growth of the bacteria, owing to the increase of osmotic pressure due to the accumulation of the products of their activity or to some specific toxic action of these products and the using up of the nutritive material. A specimen of the blood at this stage was diluted with its own volume of water so as to diminish the concentration of the waste-products, and a renewed period of growth, indicated by a steadily mounting electrical conductivity, was again observed. It may seem strange that bacteria should be able to grow at all in a liquid whose osmotic pressure is 8 or 10 times as great as that of normal blood-serum or the cell-sap of ordinary vegetable cells. But Wladimiroff * has shown that the osmotic pressure in the interior of bacteria is extraordinarily great, more than 3 times that of the cells of the higher animals. And if the bacterial cell-wall is freely permeable to any of

* *Zeitsch. f. physikalische Chemie*, vii, 529.

the decomposition products, as, for example, the red blood corpuscles are to urea, these products, although they would diminish the freezing point would not exert any osmotic pressure on the cell-wall.

Experiments III and IV show the result of observations on the growth of B. subtilis, B. proteus Zenkeri, B. proteus vulgaris, B. Friedländer, B. coli communis, Bacillus of hog-cholera, and B. lactis aërogenes. The cultures were all grown in a solution of Witte's peptone. Plates VII and VIII represent in graphic form the results of Experiment III.

As to the nature of the electrolytes produced by the bacteria, we know already numerous bodies, especially among the decomposition products of putrefying proteids, which are capable of acting as ions, for example, such organic kations as the amines and ammonium, and such anions as the acids of the fatty series.

I shall not further discuss these results at present. They are enough to show that in such observations we have a practical method of measuring the amount of decomposition produced by, and therefore the rate of growth of, bacteria. It is possible that sufficiently great and constant differences might be observed between different kinds of bacteria when grown in the same or in different liquid media to enable us to use the method as a supplement to our present means of diagnosing between nearly related forms. And for this purpose, since the curves of concentration and conductivity run practically parallel to each other, it may be that measurements of the electrical conductivity alone might suffice. These would have the great advantage over measurements of the freezing point that they could be carried out in tubes furnished with platinum electrodes fused through the glass, or perhaps passing through the plug, and the necessity of opening the tubes would thus be obviated.

It is hoped that further observations, now in progress, may cast light upon this and some of the other interesting questions arising out of this research.

Tabulated Results.

The conductivity (λ) is given in reciprocal ohms $\times 10^8$ and reduced to 5° C. In the column headed Δ the lowering of the freezing point is given in degrees centigrade.

EXPERIMENT I.—March 30, 1898. PUTREFACTION.

		Δ.	$\lambda_{(5°)} \times 10^8$.				Δ.	$\lambda_{(5°)} \times 10^8$.	
Mar. 30.	Dog's defibrinated blood (fr.). (Sp. gr. 1057.0; ash 1.544%.)	.628	33.80	Air temperature.	Mar. 30.	Serum from clot of same blood. (Sp. gr. 1022.3; ash 0.925%.)	.628	82.44	
Apr. 2.			34.16	" "					
" 6.			31.41	" "					
" 11.			28.35	" "	Apr. 2.				
Mar. 30.	Same dog's defib. blood (fresh).	.628	33.80	" "	" 5.				Put in bath at 37°–39° C.
Apr. 2.			34.16	" "	" 6.		.759		
" 5.		.632		Now put in bath at 37°–39° C.	" 8.		.834	97.99	
" 6.			55.47		" 11.		.832	104.24	
" 8.		1.271	81.77	At 2.30 p. m.	" 16.		1.308	150.63	
" 8.			83.25	At 5.25 p. m.	" 25.		2.229	220.43	
" 11.		2.043	130.00		May 15.		2.652	232.57	
" 13.		2.784	154.10				2.814	277.05	
" 16.		3.506	202.97						
" 25.		7.417	337.10						
" 27.			342.57						
May 15.		8.1 (?)	371.15						
Apr. 25.	The defibrinated blood which had been kept in bath + 1 volume water.	3.072	232.57						
May 15.	" "	4.234	335.76	Kept in bath since Apr. 25.					
" 15.	The defibrinated blood which had been kept in bath + 3 volumes water.	1.974	218.53						

EXPERIMENT II.

Jan. 4, 1898.

PUTREFACTION. DOG'S DEFIBRINATED BLOOD. THREE SAMPLES, A, B AND C, FROM THE SAME DOG. A AND B IN STERILE FLASKS; C, NOT.

	$\lambda_{(5°)} \times 10^8$. A.	$\lambda_{(5°)} \times 10^8$. B.	$\lambda_{(5°)} \times 10^8$. C.	
Jan. 4.			42.82	
" 9.	56.58		37.61	A was opened for the first time to-day, and is laked.
" 11.	60.21		45.02	A and C are both completely laked.
" 13.	65.67	63.66	48.32	B was opened for the first time to-day, and is completely laked.
" 15.	75.44	66.09	58.01	
" 20.	90.21	79.96	68.88	
" 28.	101.81	93.12	77.28	
Feb. 1.	126.75			A was put in bath at 30°–36° C. 30 hours before this measurement.
" 4.	128.68	99.41	82.71	A, B and C were put in bath at 35° C. to-day.
" 5.	139.64		90.37	
" 7.	183.70	143.47	102.80	Temperature of bath now raised to 40°–43° C.
" 8.	232.57	162.50	115.75	
" 9.	274.05	178.52	124.45	
" 10.	308.02	214.47	147.65	
" 16.	359.58	314.13	232.57	
" 24.	356.04			
" 25.	359.58	381.78	318.87	
" 26.	363.70	390.00		
" 28.	364.26	393.02	338.00	
Mar. 2.	360.09	397.96	343.03	
" 3.		403.02	345.37	
" 7.	359.07	397.96	347.26	
" 11.	349.65			
" 22.		399.85	340.27	
Apr. 27.	382.93	396.09	343.03	

From Jan. 4 to Jan. 31, A, B and C were all kept at room temperature. When A and B were first opened (on Jan. 9 and 13, respectively) neither was found completely sterile. A rod-shaped bacterium was present in both, but there was no putrid odor, although this was very marked in C. After being opened A and B were thoroughly exposed to the air and allowed to putrefy like C.

EXPERIMENT III.

A STERILE SOLUTION OF WITTE'S PEPTONE WAS PREPARED. ON APRIL 11, 1898, 6 TUBES WERE INOCULATED WITH BACILLUS SUBTILIS AND 4 TUBES WITH BACILLUS PROTEUS ZENKERI.

		Δ.	$\lambda_{(5°)} \times 10^5$.	
1898. Apr. 5.	Sterile peptone solution.	.124	13.68	Ash .0876 grm. Solids 4.3052 grm. } in 100 cc.
" 11.	Sterile peptone solution (after further sterilization in steam sterilizer.)	.101	11.25	
" 11. 1 p. m.	Inoculated 6 test-tubes with B. subtilis and 4 with B. proteus Zenkeri.			
" 13. 4 p. m.	B. subtilis No. 1.	.104	12.12	At 35° C. since inoculation. Considerable growth.
	B. proteus Zenkeri No. 1.	.114	11.53	At 35° C. Less growth.
" 16.	B. subtilis No. 2.	.121	13.65	At 35° C. for 3 days; then room temperature since.
	B. proteus Zenkeri No. 4.	.104	12.27	" "
" 27.	B. subtilis No. 3.	.152	16.42	In bath at 40° C. since April 20.
May 15.	B. subtilis No. 4.	.571	51.57	In bath at 40° C. since April 27.
" 15.	B. proteus Zenkeri No. 3.	.134	13.37	At room temperature.
" 15.	B. proteus Zenkeri No. 2.	.125	12.56	At room temperature. Very little growth.
" 15.	B. subtilis No. 5.	.231	21.91	At room temperature since inoculation. Fair growth.
" 15.	B. subtilis No. 6.	.219	19.03	At room temperature since inoculation. Fair growth.
Apr. 13.	B. subtilis No. 1.	.104	12.12	Now exposed to air and put in bath at 40° C.
" 16.	B. subtilis No. 1.	.589	65.57	Far more growth than in unopened tubes.
" 25.	B. subtilis No. 1.	1.267	144.45	
May 15.	B. subtilis No. 1.	1.482	171.05	
" 15.	B. subtilis No. 3.	1.197	142.98	Exposed to air on April 27. In bath at 40° C. since.
Apr. 13	B. proteus Zenkeri No. 1.	.114	11.53	Exposed to air after opening and put in bath at 40° C.
" 16.	B. proteus Zenkeri No. 1.	.530	59.97	
" 25.	B. proteus Zenkeri No. 1.	1.093	132.86	

EXPERIMENT IV.—April 18, 1898, 1 P. M.

TWO TUBES OF THE SAME SOLUTION OF WITTE'S PEPTONE WERE INOCULATED WITH CULTURES OF THE FOLLOWING BACTERIA. ALL WERE PUT IN THERMOSTAT AT 35° C., EXCEPT B. SUBTILIS AND B. PROTEUS VULGARIS, WHICH WERE LEFT AT ROOM TEMPERATURE (10°–15° C.). BUT ON APRIL 27 B. SUBTILIS WAS PUT IN BATH AT 40° AND KEPT THERE TILL MAY 15.

	B. subtilis.		B. proteus vulgaris.		B. Friedländer.		B. coli communis.		B. of hog-cholera.		B. lactis aërogenes.	
	Δ.	$\lambda_{(5°)} \times 10^8$.	Δ.	$\lambda_{(5°)} \times 10^8$.	Δ.	$\lambda_{(5°)} \times 10^8$.	Δ.	$\lambda_{(5°)} \times 10^8$.	Δ.	$\lambda_{(5°)} \times 10^8$.	Δ.	$\lambda_{(5°)} \times 10^8$.
Apr. 22, 2.35 p.m.					.154	17.95	.181	16.98	.142	17.40	.181	20.76
Apr. 27, 11.50 a.m.			.156	20.15								
May 12.					.207	20.05	.187	19.69	.167	16.52	.246	25.59
May 15.	.318	36.31	1.343*	158.52								

Control peptone tube in thermostat till May 12, when $\Delta = .148$ and $\lambda_{(5°)} \times 10^8 = 16.10$.

* Test-tube opened on April 27 and left in bath at 40° C. with ordinary cork till May 15.

DESCRIPTION OF PLATES V-VIII.

PLATE V.

Curves plotted from the results of Experiment I to show the changes in the molecular concentration of putrefying blood and serum.

FB, freezing point of blood; *FS* of serum; *CB*, conductivity of blood; *CS* of serum. *A* shows the course of the curve of conductivity in the defibrinated blood kept at air temperature. On April 2 the blood and serum were put in a bath, the temperature of which varied between 37° and 39°.

PLATE VI.

Curves plotted from the results of Experiment II to show the changes in the electrical conductivity of putrefying blood.

A, *B*, *C*, curves of three samples of blood taken at the same time from the carotid of a dog.

a shows the point at which (on Jan. 28) *A* was put into bath at 30°-36° *C*; *b*, the point at which (on Feb. 1) it was taken out of the bath. The dotted line passing through the three curves and labelled "Bath" shows the point where (on Feb. 4) *A*, *B* and *C* were put into bath at 35°, where they were afterwards kept. *I* is a curve from a specimen of blood from another dog, the numerical results of which are not quoted.

PLATE VII.

Shows the changes in the conductivity of the contents of five tubes (Nos. 1, 3, 4, 5 and 6) inoculated with Bacillus subtilis on April 11. The curves are plotted from the results of Experiment III.

PLATE VIII.

Shows the changes in the freezing point of the contents of the same tubes.

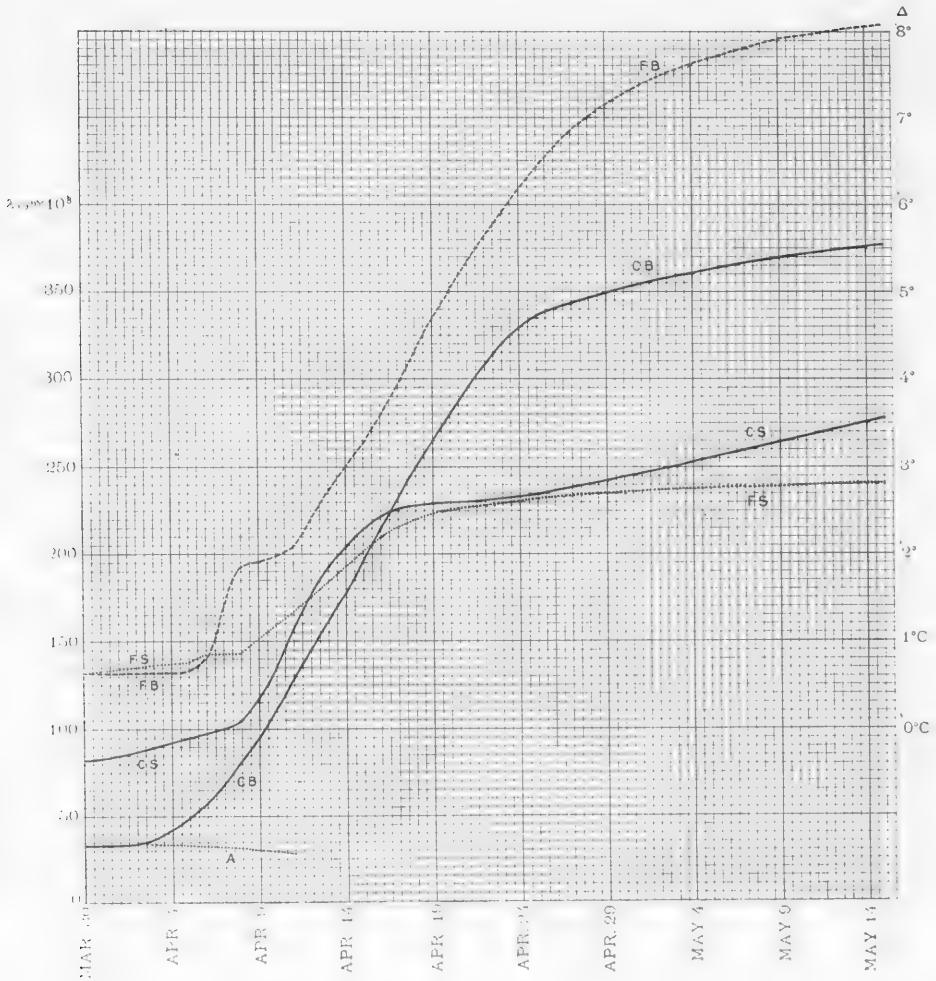
On April 13, No. 1 was freely exposed to the air and then put in a bath at 40°, corked tightly with an ordinary cork.

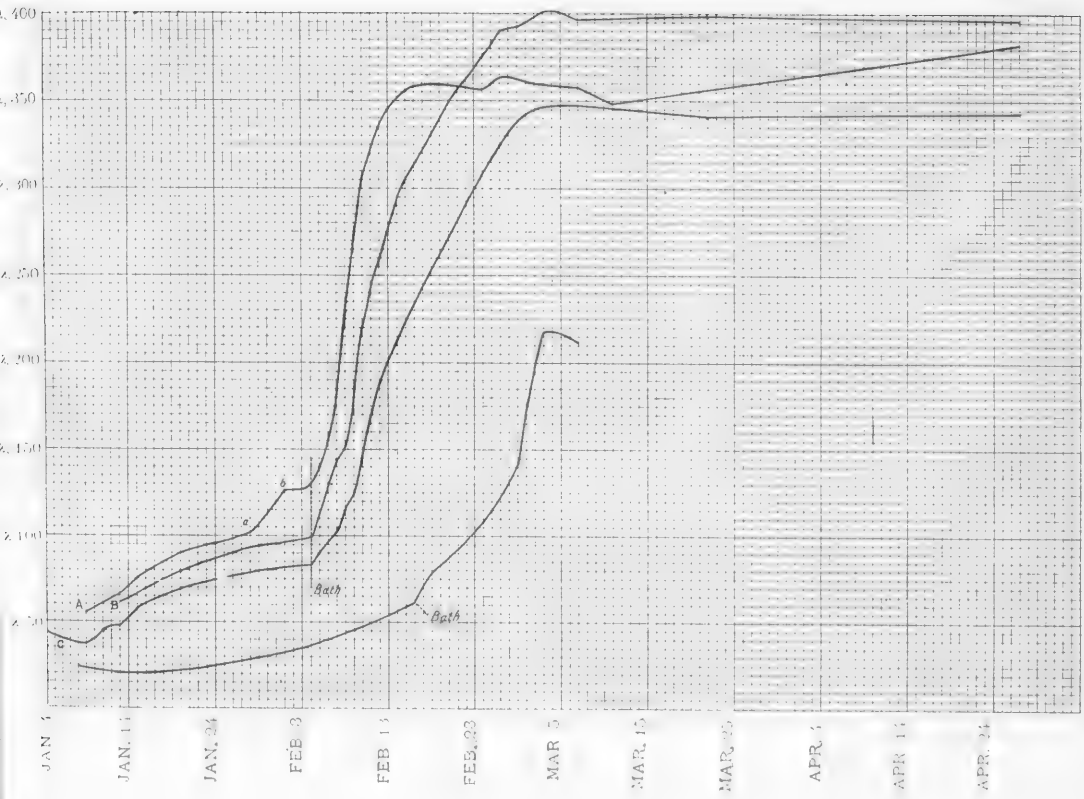
On April 27, No. 3 was treated in the same way.

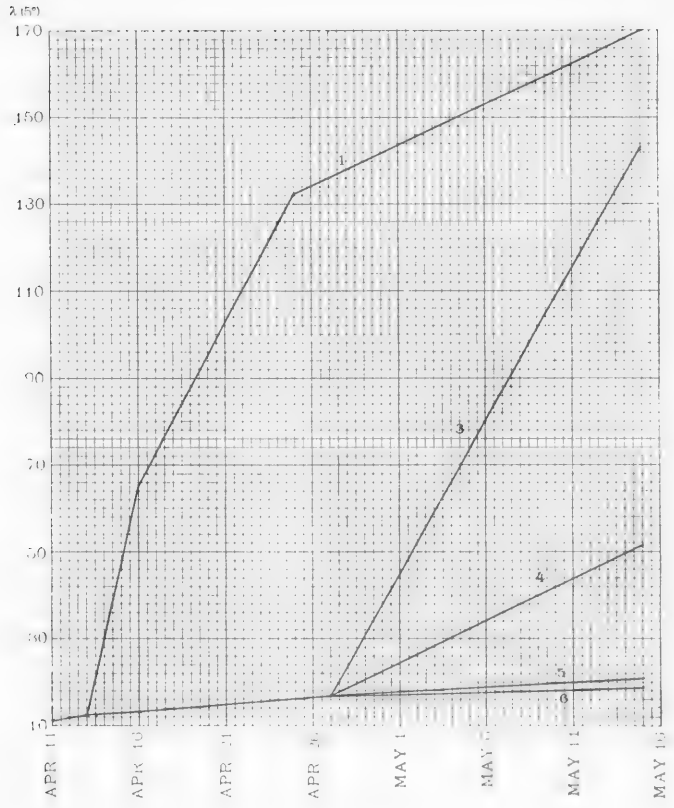
No. 4 was put in the bath at 40° on April 27 and kept there till May 15, when it was opened.

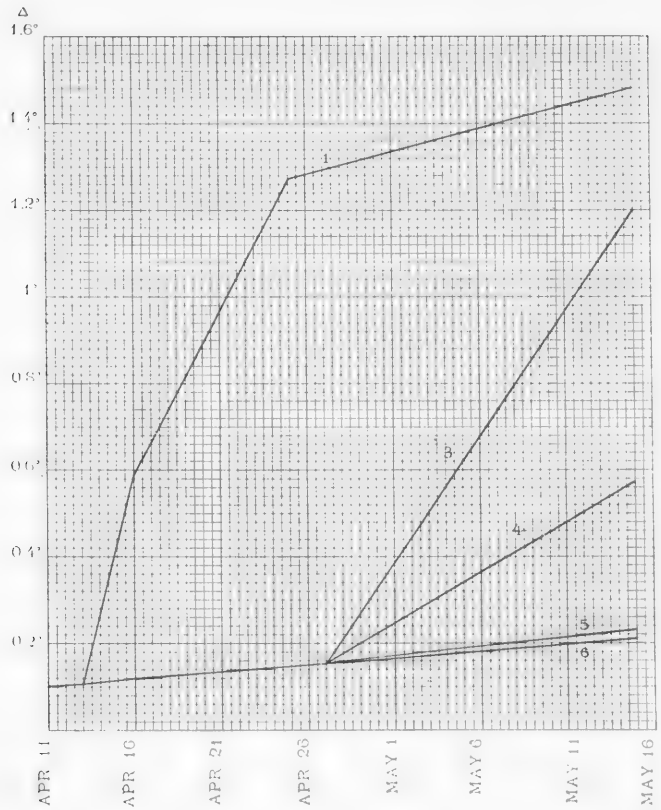
Nos. 5 and 6 were kept at the room temperature.

The curves of conductivity (Plate VII) and molecular concentration, it will be seen, run nearly parallel to each other.


Δ
8°
7°
6°
5°
4°
3°
2°
1°C
0°C
350
300
250
200
150
100
50
0
FB
CB
CS
FS
A
APR. 29
MAY 4
MAY 9


400
350
300
250
200
150
100
50
A
B
a
a'
a''
Bath
Bath
JAN. 1
JAN. 14
JAN. 24
FEB. 3
FEB. 13
FEB. 23
MAR. 5
MAR. 15
MAR. 25
APR. 4
APR. 14
APR. 24


λ(cc)
170
150
130
110
90
70
50
30
10
1
3
4
5
6
APR
APR
APR
APR
MAY
MAY
MAY
MAY


Δ
1.6°
1.4°
1.2°
1°
0.8°
0.6°
0.4°
0.2°
APR 11
APR 16
APR 21
APR 26
MAY 1
MAY 6
MAY 11
MAY 16
1
2
3
4
5
6

ON SUPRA-ARTERIAL EPICARDIAL FIBROID NODULES.

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PLATES IX–XI.

The morbid condition to be described in this article is characterized by the presence of multiple, grayish-white, fibroid nodules situated upon the coronary arteries of the heart. Although similar nodules have doubtless been previously observed, I have been unable to find any satisfactory description of them in medical literature. I have, therefore, taken advantage of the opportunity to study histologically five well-marked examples which, during the last few months, have been observed among the autopsies at the Johns Hopkins Hospital.

These supra-arterial nodules differ from the ordinary tendinous or milky patches, often observed upon the epicardium, especially over the right ventricle, in their multiplicity, their smaller size, and their distribution over the coronary arteries to which they evidently bear some definite relationship. In gross appearance they resemble much more closely the nodular affection of the arteries first described by Kussmaul and Maier* in 1866 under the name "periarteritis nodosa." In consequence of this resemblance, which, however, is only superficial and does not pertain to the histological structure, it is appropriate to direct attention to the latter affection.

Kussmaul and Maier observed nodular thickenings on all the smaller arteries of the body, except those of the central nervous system, in a man aged 27 who, after leading a vagabond's life, entered the hospital at Freiburg complaining of weakness and muscular pains. He grew gradually worse, had an intermittent temperature, suffered from gastro-intestinal disturbances and, late in his illness, with a progressive paralysis

* *Deutsches Arch. f. klin. Med.*, 1866, i, 484.

which showed from time to time short remissions. There were also symptoms of nephritis. He was ill four weeks.

At autopsy the most noticeable feature was the presence of countless opaque nodes on the smaller arteries of the body. The pericardium was smooth except where the coronary arteries were studded with these uniform grayish-white patches.

Microscopical examination showed usually no changes in the intima of the arteries, but there was a cellular infiltration and degeneration of the media, with infiltration and much hypertrophy of the adventitial coat, the lumen of the vessel being usually diminished, but occasionally dilated. Kussmaul and Maier expressed the opinion that the arterial changes were primary, and that the condition was a hitherto undescribed affection characterized by well-marked symptoms. They had no suggestion to make as to the cause of the arterial changes.

Chvostek and Weichselbaum * reported in 1877 the case of a soldier, aged 23, who became suddenly ill, complaining of headache, dizziness and nausea. There followed irregularity of the pupils and paralysis of the left ocular nerve, of the right side of the face and of the right extremities. He died after an illness of four months.

On section a ruptured aneurism of the left arteria profunda cerebri (posterior cerebral) was found, with hæmorrhage into the cortex. Many of the medium-sized and small arteries of the body, but only at their bifurcation, were the seat of opaque gray swellings varying in size and shape. There were caseous areas in the lungs, pleuræ, liver and kidneys.

Microscopical examination of some of the arterial tumefactions showed an increase in the cells of the intima with small-celled infiltration. These changes extended through the media and met a like proliferation of the adventitial coat, the elastic tissue offering the most resistance. There were aneurismal dilatations of some of the affected arteries. The condition was primarily, they consider, an endarteritis of luetic origin resembling that described by Heubner.† There were no other suspicious lesions nor was there a syphilitic history.

A somewhat similar case is described by P. Meyer.‡ A soldier, 24 years of age, after a life of dissipation, was admitted to a hospital complaining of great muscular pain, headache, sleeplessness, irregular fever and weakness. These symptoms became more marked and death took place in two months.

* *Allg. Wiener med. Zeitung*, 1877, xxii, 257 *et seq.*

† Die leutische Erkrankung der Hirnarterien, etc., Leipzig, 1374.

‡ Virchow's *Archiv*, 1878, lxxiv, 277.

The smaller arteries of the body were found at autopsy to be the seat of several yellowish-white dilatations from a mustard seed to a pea in size, most frequently situated at the branching of the artery involved. The vessels of the brain and cord were quite normal. The primary change Meyer conceives to consist in the rupture of the elastic coat of the artery leading to a dilatation of the other layers with thrombus formation and subsequent proliferation of the arterial walls.

He thinks that sudden changes in blood pressure from the ingestion of large quantities of fluid, from great exertion and from dissipation had weakened and broken through the strong elastic fibres with the above-mentioned secondary results.

This view is supported in part by Eppinger,* who brings the affection into line with aneurisms produced by increased blood pressure acting upon arteries whose elastic tissue is in places congenitally weak, and producing a bowing out of the wall with secondary hypertrophy of the intimal and adventitial layers.

The cases thus far have been those of young men, but that the process is not limited to this class is shown by the report of Fletcher.† A widow, aged 49, presented herself at the hospital complaining of muscular pain and weakness with occasional swelling of the legs. Later there were also intermittent fever, cough and albuminuria. The diagnosis rested between typhoid fever and miliary tuberculosis. Death occurred after two months.

The anatomical findings were very similar to those of von Kahlden,‡ who, in 1894, reported two cases in which like alterations in the arteries were observed. Both patients were women beyond middle life, 49 and 52 years of age, the latter the mother of five normal children. In each case the symptoms were increasing weakness and fever to which in the older woman were added great pain and gastro-intestinal disturbance. The duration of the illness was 8 and 12 weeks respectively. On section, each of these three cases showed upon the small and medium-sized arteries circumscribed whitish nodules, most abundant in the heart, pericardium, mesentery, pleuræ, liver and kidneys, the arteries of the central nervous system being unaffected.

Fletcher and von Kahlden agree with Chvostek and Weichselbaum in considering that the process begins by a proliferation of the intima followed shortly afterwards by a like process with leucocytic infiltration in

* Pathogenesis, Histogenesis u. Aetiologie der Aneurysmen, Berlin, 1887.
† Zeigler's *Beiträge*, 1891, xi, 323.
‡ Zeigler's *Beiträge*, 1894, xv, 587.

the adventitial layer. This cellular tissue, they assert, invades the media on both sides leading to its rupture and to the degeneration of both muscular and elastic tissues, with an accumulation of small round cells and fibrin; a thrombus filling the lumen in the later stages completes the picture. They do not accept the hypothesis that the affection is due to syphilis on account of the complete absence of luetic history and lesions, but they are disposed to consider the causal agent to be some other, bacterial or toxic, infection of the blood which attacks the internal coat of the vessels and produces those changes which they consider to be primary.

From this brief review there would appear to be several reported instances of an affection of the smaller arteries, characterized macroscopically by circumscribed whitish nodules distributed pretty generally through the body, except in the central nervous system, and microscopically by hypertrophy of both internal (except in the case of Kussmaul and Maier) and adventitial coats with weakening, and, in places, with rupture of the elastic coat. There was sometimes dilatation of the vessel, but usually the lumen was narrowed.

The lesions in these cases were accompanied by clinical symptoms having much in common. The onset was sudden and marked by muscular pain, weakness, intermittent fever and gastro-intestinal disturbances. There were occasionally also subsequent paralysis, anæmia and nephritis. The course of the disease was progressively fatal, death resulting in all cases in from 7 to 12 weeks.

Three suggestions have been made as to the etiology. Chvostek and Weichselbaum reasoning from the close resemblance to arterial changes of known syphilitic origin, consider lues to be the cause of the lesions. Meyer and Eppinger think that the primary change is a weakening of the elastic membrane; while Fletcher and von Kahlden are of the opinion that the proliferation of the intima is the first step, and that this is brought about by the direct action of bacteria or of toxins.

The gross appearance of the nodules on the surface of the hearts now under consideration agrees closely with that of the arterial thickenings scattered generally through the body in the cases of so-called periarteritis nodosa.

Description of gross specimens (Plate XI). In the specimens examined the extent of the process varied much, from tortuous, more or less uniform, elevations over the arteries, to whitish dots minute in size and few in number which almost escape attention. In most of the specimens the epicardium between the arteries was delicate, the only superficial lesion, other than an occasional "milky patch," being the grayish-white nodules along the smaller arteries. These elevations may be separated from each other by wide intervals or they may be quite close, resembling beads strung along the vessel, or they may coalesce throughout the entire length of the artery (Case III).* They hold no demonstrable relationship to the points of bifurcation of the vessels, nor was any particular branch especially affected. In no case did the thickening tend to encircle the artery. In one case (Case V) they were noticed, not only over the ventricle, but also, although few in number, upon the auricle, and over the small vessels in the adventitia of the aorta in its ascending part, where a single small focus measuring 1x2 mm. in diameter existed. In the same case a few scattered nodules were present also over the large veins (Plate XI). There was no tendency to aneurismal formation.

Microscopical.—The appearances observed under the microscope differ somewhat in different cases, although the main lesion is, as I hope to show, constant. In Cases I, II, III and IV the following conditions were observed in cross-sections including the coronary arteries with the nodules upon their adventitial surfaces. The intima of the arteries in the majority of sections examined showed little alteration; there may be slight swelling and the inner margin may show a somewhat irregular surface, which is not excluded from being the result of irregular contractions of this coat in the hardening process. In several specimens there could be made out a marked increase in the intimal layer, either as a single mass bulging into the lumen, representing perhaps an organized mural thrombus or as several smaller, more diffuse protrusions consisting of fusiform and branched cells with some intercellular substance. In the last case examined (Case V) the intimal changes are much more pronounced, being most marked in situations corresponding to the nodes. The entire internal coat is thickened, but the increase is especially marked in the myocardial hemispherical segment of the artery. In this situation it is fully six times its normal thickness and the muscular tunic is here somewhat diminished and degenerated. The increase in the thickness of the intima on the epicardial side is relatively slight, although the muscle in this region is degenerated and attenuated to a greater degree than on the opposite side of the vessel.

* Protocols of the cases are appended to this article, pp. 257–260.

The muscular coat (media) is in the main, except in the instance to be presently mentioned, unchanged, so far as can be determined, in the sections stained by hæmatoxylin and eosin. Occasionally there is an apparent degeneration of muscular fibres as shown in a reduction in number of muscle-cell nuclei and there is exceptionally an infiltration with small round cells. In Case V, as already mentioned, the media shows much more pronounced pathological changes, in that this tunic is reduced in thickness and areas of hyaline metamorphosis are apparent.

The changes in the adventitia proper are slight and inconstant. Only occasionally is there any hypertrophy.

The nodular formations lie upon the vessels within the epicardium, being seated primarily in the layer of connective tissue between the endothelial covering and the delicate layer of elastic fibres which rests upon the main layer of loose vascular connective tissue containing the epicardial fat. Their situation corresponds, therefore, to that of milky patches as determined by Ribbert.* In their immediate neighborhood are found the usual loose adipose and connective tissues, vessels and nerves. But the nodules differ from the normal connective tissue, being at once distinguished from this through their dense and fibrous, often sclerotic, appearance.

The appearance on cross-section is as though a compact mass of firm connective tissue, convex on its inner surface, were set upon the artery in the loose epicardial tissue. The lateral edges of the thickening slant gradually upward to the surface and are continuous there with the epicardium (Plates IX and X). Scattered through the firm mass are a few fusiform connective-tissue cells. At the base of the area and at the sides are often groups of lymphocytes and sometimes a small number of polymorphonuclear leucocytes (Plate IX, Fig. 1 and Plate X, Fig. 1). The firm tissue pushes up beyond the level of the rest of the epicardium and forms the opaque nodule seen in the gross specimen. The endothelium and occasionally some subjacent tissue cover the prominence and are continuous with the serosa over the rest of the heart. Beneath the nodules the layer of epicardial elastic tissue can usually be demonstrated, but the subjacent loose connective and adipose tissues are more or less atrophied.

The size of the fibrous thickening varies within wide limits, both in depth and in lateral extension. It may form a comparatively narrow band which is not raised above the surface of the epicardium; or, again, it may appear as a high, irregular, almost pediculated projection rising from a compact base above the vessel (Plate X, Fig. 2).

* Virchow's *Archiv*, 1897, cxlvii, 211.

The nodule may not only cover the outer surface of the vessel but may extend a considerable distance in the loose cellular tissue on each side (Plate X); or, on the other hand, there may be an oval patch over only a portion of an artery, the remaining peri-adventitial covering being quite normal (Plate IX, Fig. 1).

The early stages of the process leading to the supra-arterial nodules presented a tissue richer in cells, both fibroblasts and lymphoid cells, situated superficially to the vessel and on its epicardial side. As the nodule becomes older, the more homogeneous and less cellular becomes its structure; the nuclei are fewer and the focus is more sharply differentiated from the surrounding tissue. In no instance was any tendency to a similar fibrous formation noticed on the side of the affected vessel next to the heart muscle, nor were any similar alterations seen about the arteries in the substance of the myocardium.

We have, then, in these cases, nodules on the surface of the heart macroscopically resembling those described by Kussmaul and Maier but totally dissimilar in their minute structure and relations; for the specimens stained in hæmatoxylin and eosin failed completely to show any constant degeneration or proliferation of the arterial walls, such as is present in periarteritis nodosa. The uniform relationship to arteries, however, suggested that there was probably some alteration in the vessel which was at least associated with the nodule. Hence a representative number of the sections were stained for elastic tissue by the fuchsin method of Manchot * and by the method of Weigert†.

Results of stain for elastic tissue. There were of course variations in the sections, but the general results were sufficiently uniform to be quite suggestive. The stains brought out the yellow elastic fibres in dark-red or violet, in contrast to the pale, partly decolorized tissues. In a few of the sections there were distinct breaks in the inner elastic coat just opposite the nodule.

The most noticeable alteration, however, and one present in two-thirds of the specimens was a diminution in the strength of the *outer* elastic coat between the muscle and adventitial layers. This membrane was well represented in the inner (myocardial) side of the artery, often by a heavy dark band, but toward the outer (epicardial) half it became

* Virchow's *Archiv*, 1890, cxxi, 111.

† *Centralbl. f. allg. Path.*, 1898, ix, 289.

thinned, the fibres appeared looser and separated and more or less interlaced by the coarser bands of the adventitial coat until, beneath the thickening over the vessel, perhaps the last trace of the elastic membrane disappeared (Plate IX, Fig. 2).

In no instance was this change accompanied by an increase in the thickness of the inner elastic layer; in fact, at times the latter also appeared reduced.

The extent of this reduction or disappearance of the outer elastic coat differed widely. The thinning of the elastica was often present not only beneath the fibroid nodule, but extended a considerable distance in each direction, affecting perhaps half the circumference; or, again, it was more localized and perhaps somewhat to one side of the thickest part of the node (Plate X, Fig. 2).

The normal relative strength of the inner and outer elastic layers varies considerably; usually the inner coat is the stronger and the fibres seem more compact, but in other specimens it appears to be the weaker of the two and the main elasticity of the vessel wall is evidently supplied by an outer membrane which is thick and firm. In a rupture of this strong band there is usually not a simple break in the continuity of its fibres, but they split up into a perfect meshwork, spreading through the adventitia (Plate X, Fig. 2). Elastic fibres were made out in some instances in the nodule, more commonly as a definite layer between it and the underlying connective tissue (Plate IX, Fig. 2). Occasionally there was a proliferation of elastic fibres through the thickened intima. Only rarely were defects in either elastic coat seen in the inner (myocardial) side of an artery. In these cases no alteration corresponding to the nodule under discussion was noticed in the remaining layers nor in the surrounding tissue. No intrinsic changes in the elastic fibres suggesting degeneration were made out; the only microscopical deviation from the normal was the apparent mechanical separation and the disappearance of the fibres. Several sections containing arteries not surmounted by nodules were stained in the same manner, and although irregularities in the elastic coats were found, there was not in any case the marked alteration described in the elastic membrane as existing beneath the fibrous thickenings.

Sections of the diffuse areas of epicardial thickening, known as "milky patches," showed the appearances described by Ribbert.* No relationship of these to blood-vessels was observed.

* Virchow's *Archiv*, 1897, cxlvii, 207.

The foregoing microscopical observations indicate a relationship between the fibroid nodules and the demonstrated weakening in that part of the arterial wall immediately beneath the nodule.

In considering the etiology of this certainly distinct lesion, one must recall the diseases with which it may be associated.

(1) There was a distinct history of syphilis in one case (III); in three it was denied (I, II and V), while there is reason to be quite sure that there was no luetic infection in the fifth case (IV). The nature of the histological changes does not correspond to that of a syphilitic affection. The arterial alterations produced by lues consist in an endarteritis associated usually with a periarteritis, or the direct inclusion of the vessel in surrounding gummatous material. In the sections examined the arterial walls were unaffected, except in the manner already described, and in no instance were caseous areas seen in the adjacent tissues.

(2) As far as the gross appearances go, the nodules might possibly be mistaken for tubercles. One of the cases reported had suffered from a general tuberculous peritonitis (Case III) for which laparotomy had been performed. In the other cases there was no evidence whatever, clinical or anatomical, of infection with the tubercle bacillus nor was there the slightest sign of any tuberculous structure in any of the preparations.

(3) It will be recalled that blood infection from either bacteria or their toxins was the cause suggested for periarteritis nodosa by the later writers, Fletcher and von Kahlden. They based their hypothesis upon the primary proliferation of the intima which they attributed to the direct action of toxic substances in the circulation. This cellular increase in the intima, as has been shown, is conspicuous chiefly by its absence in most of the sections from our cases, although it may be present, as in Case V. Moreover, cultures from the heart's blood were negative in two instances, while Staphylococcus pyogenes albus alone was found once, as was also in one case Staphylococcus pyogenes aureus. In the fifth case blood cultures taken during life remained sterile. It would be quite impossible to imagine that the nodules could be due to poisonous agents carried in the blood stream

and producing this fibrous thickening on but one side of the artery, while they so rarely affect a vein and do not injure further the intermediate arterial coats.

We may, therefore, doubtless exclude syphilis, tuberculosis and other infections as essential etiological factors in the production of these supra-arterial nodules.

The evidence seems to me strongly to support the view that the primary, underlying cause is to be found in a weakening of the arterial wall, due usually to defects in one or both of the principal elastic lamellæ of the artery, most frequently of the elastica externa. A number of facts in the protocols lend color to this theory. All the cases were in males between 19 and 54 years of age, accustomed, with one exception (Case III), to hard work, irregular methods of life, indifferent nourishment and varying quantities of alcoholic beverages. The heart was hypertrophied in four cases (I, II, IV, V); in one (IV) there were valvular lesions, in two (II and V) arteriosclerosis, in four (I, II, III, V) nephritis, in four (II, III, IV, V) œdema, and in a single case (I) aneurisms existed. These conditions indicate that during life there must have been irregularities in the force of the blood pressure, influenced further, doubtless, by the ingestion of large quantities of fluid.

The duration of the final illness varied from 8 weeks to 2 years. So far as known, no symptoms are attributable to the epicardial nodules.

As a probable explanation, then, of the origin of these fibroid nodules it is suggested that there is a weakening of the arterial coats, mainly of the outer elastic coat, on the side toward the nodule. While this defect may be congenital, it is more probable that it is acquired through poor nutrition combined with sudden alterations in blood pressure from the causes already indicated. This loss of elasticity, it may be supposed, is compensated by a fibroid thickening, situated not in the vessel wall, but beyond it in the epicardial tissue.

For such an explanation an analogy is to be found in the views now generally held of the formation of aneurisms, first advanced by Rokitansky and later supported, with certain minor modifications, by

Eppinger, P. Meyer, Manchot, Thoma and others. These authorities consider the primary change to be a giving away of the media, especially of the elastic laminæ and fibres, with subsequent bulging out of the arterial wall. When the weakening takes place slowly, cellular proliferation occurs in the intima and adventitial coats and the danger of aneurism is lessened by the thickening of the arterial wall. Tears in the elastic lamellæ are not uncommon, and may occur without the formation of aneurism. The high pressure in the coronary arteries would seem to render these vessels particularly exposed to such injuries, when the nutrition of their walls is impaired. Of especial interest in our cases is the demonstration of more frequent and pronounced defects in the outer elastic lamella than in the internal one.

It will be recalled that in no case was there an increase in the surrounding connective tissue corresponding to the inner or myocardial side of the artery and it is to be conjectured that here the heart muscle affords sufficient support to prevent stretching of the vessel walls with such ruptures of the elastic lamellæ as were observed on this side. Each pulsation must produce expansion of the artery, which is most marked at the point of least resistance, *i. e.* at the outer or epicardial surface. Excessive or irregular expansion can easily be thought of as injuring more or less the elastic coat. In such cases there follows a still greater protrusion outwards of the vessel wall with each pulsation. The significant localization of the defects in the outer elastic lamellæ upon the epicardial side of the arteries indicates the greater exposure of the artery upon this side to injury, and this may be due to the lack of the support which is afforded to the myocardial side of the artery by the surrounding tissues. The firm resistance offered to the expansion of the vessel on the inner side by the ventricular wall is an important factor in favoring increased bulging in the free semi-circumference. The absence of this counter-support probably accounts for the fact that similar nodules are rare upon the auricles.

SUMMARY AND CONCLUSIONS.

1. Fibroid nodules seated in the epicardium directly over branches of the coronary arteries of the heart are not uncommon. They may be present in large numbers and are found most frequently upon the

surface of the ventricles, but may occur over the auricles and even on the outer surface of the ascending aorta. They are rarely observed over the coronary veins.

2. While often resembling in gross and superficial appearances the nodules described by various writers under the name of "periarteritis nodosa," they differ from these in essential respects. They are seated outside of the adventitial coat and lie within the epicardium. They are composed of dense, fibrous, sclerotic tissue, poor in cells. In earlier stages of their formation they are richer in cells, both fibroblasts and lymphoid cells.

3. These supra-arterial nodules bear no definite relation to endarteritis, although they may be associated with this condition.

4. There were found with great regularity in the arterial wall immediately beneath the nodule, changes, which indicated a weakening of the wall in this situation. In some instances the muscular coat was thinned and degenerated, but the most common and important change was reduction and often disappearance of the elastic lamellæ and fibres, the outer elastic lamella being the one most frequently and intensely affected. These lesions were often limited to the segment of the arterial wall adjacent to the epicardium, the inner or myocardial segment of the same artery being free from similar alterations, or presenting them only in a slight degree. It is suggested that the absence on the outer or epicardial segment of the firm support afforded to the artery on the inner or myocardial aspect by the surrounding tissues renders the former more liable to damage to the elastic tissue resulting from irregularities and increase of blood pressure associated perhaps with defects of nutrition.

5. In consequence of the weakening in the arterial wall the artery would tend to bulge at the affected spot toward the epicardium were this tendency not restrained. The formation of the dense supra-arterial nodule of fibrous tissue over the weakened area holds this tendency in check and may therefore be regarded as an adaptive or compensatory change.

The question as to the immediate exciting cause of the new growth of tissue offers the same difficulties as that pertaining in general to

similar growths of connective tissue. Some would doubtless attribute it to direct stimulation from the pressure and shock of the impinging artery, others to defects in the tissue, and still others to a disturbance of the neighborhood relations of the part. It is not deemed necessary to enter into a discussion of these various hypotheses.

I take pleasure in expressing my thanks to Dr. Welch for examining my sections and to Dr. Flexner for constant advice and help.

PROTOCOLS OF THE CASES.

Case I. Man, G. J., aged 45, colored. Clinical history not obtainable.

Anatomical diagnosis. Enlargement of middle lobe of prostate gland; purulent cystitis, pyoureter, pyglonephritis, pyonephrosis.

Cardiac hypertrophy without valvular lesion; supra-arterial coronary nodules. Hæmorrhage from penis and stomach. Broncho-pneumonia. Emphysema. Exostoses on ribs.

Additional notes. Pericardium shows "milk patch" over right ventricle. The remainder is smooth except along the ramifications of the branches of the coronary arteries over both ventricles, where grayish-white, semi-translucent nodules, varying in size from an ultimate tubercle to one of a millimetre in diameter exist. These may be scattered at intervals along the vessel or in the case of the flatter and more opaque ones may almost coalesce throughout the entire length of the vessel. Apparently there is no bulging inward of the vessel wall corresponding to the thickenings. The valves are delicate. The left ventricle is thickened, not dilated. Weight of heart 370 grammes. No arterial nodules detected in any of the organs.

Bacteriological report. Heart and lungs, Staph. pyog. albus.

Case II. Man, S. C., aged 54, colored, coachman.

Clinical history. Patient came to hospital complaining of shortness of breath, cough and swelling of the legs and abdomen. Has led a rough, exposed life. Used tobacco and alcohol freely. Fifteen months before admission had attack of dyspnœa, which has been repeated many times since, together with œdema of face and of extremities and ascites. Much difficulty in micturition. Urine contains albumin and hyaline casts. Died eleven days after admission, in which time 3800 cc. of fluid were drawn from his abdomen.

Anatomical diagnosis. Arteriosclerosis. Aneurism of the thoracic and abdominal aorta. Cardiac hypertrophy. Supra-arterial coronary

nodules. Chronic passive congestion of the viscera. Chronic diffuse nephritis. Atrophy and cirrhosis of liver. Œdema and ascites. Ecchymoses in the serous membranes.

Additional notes. Pericardium generally smooth. Following the course of the coronary arteries and their branches are small grayish-white nodular thickenings from a pin's head to a millet-seed in thickness. Vegetations are present on the aortic segment of the mitral valve. A bulging is seen in the transverse arch of the aorta and a second dilatation in the abdominal aorta below the superior mesenteric artery.

Bacteriological report. Cultures from heart, lungs, peritoneal cavity, liver and spleen are negative.

Case III. Man, W. D., aged 43, colored.

Clinical history. Admitted to hospital complaining of swollen abdomen and dyspnœa. One brother died of phthisis. History of syphilis, gonorrhœa and abuse of alcohol. Patient felt well until 6 weeks before admission when he began to complain of headache, muscular pain, and weakness followed by cough, dyspnœa and ascites. He was transferred to the surgical side, laparotomy was performed and a general tuberculous peritonitis was found. Death occurred 18 days after operation, about 10 weeks after the onset of symptoms.

Anatomical diagnosis. Tuberculosis of peritoneum. Tubercles in form of large caseous and conglomerate masses. Purulent peritonitis. Operation wound for laparotomy. General tuberculosis of lymphatic glands and abdominal organs. Acute tuberculosis of left pleura. Hydrothorax. Double hydroureter and hydronephrosis. Interstitial orchitis.

Additional notes. Heart small. The coronary arteries generally are marked out by grayish-white elevations fusing together over them, forming tortuous opacities. The epicardium between these thickenings is normal except for some milky patches. The intima appears smooth.

Bacteriological report. Cultures from lungs, heart, kidney and liver show Staphylococcus pyogenes aureus.

Case IV. Man, J. H., aged 19, white, florist.

Clinical history. Patient admitted to hospital complaining of cough, dyspnœa and palpitation. Three months previously had suffered with œdema of ankles, but resumed work until a month before admission, when the symptoms complained of made it necessary to stop. On admission there were marked evidences of aortic and mitral disease with loss of compensation and cardiac enlargement. He died at the end of three weeks after admission, during which time the symptoms had become worse.

Anatomical diagnosis. Proliferative endocarditis affecting aortic and mitral valves. Hypertrophy and dilatation of both sides of the heart. Relative insufficiency of mitral and tricuspid valves. Hypoplasia of aorta. Chronic passive congestion of lungs, liver, spleen and gastro-intestinal tract. Œdema, ascites, hydrothorax, jaundice.

Additional notes. Visceral pericardium smooth except for a few granules of fibrous thickening at the outer edge of auricle and over the right ventricle where there are a number of minute, rather translucent white granular spots, slightly elevated, lying over the course of vessels.

Posterior segment of chordæ tendineæ of mitral valve shortened and thickened. Aortic valve thickened along its free edge. No nodules in organs.

Bacteriological report. Cultures from peritoneal cavity, heart, spleen and mitral valve, negative.

Case V. J. R., man, aged 48, colored, upholsterer. Complained on admission of shortness of breath and swelling of legs. Had always done hard manual labor and used tobacco freely, otherwise fairly good personal history. Present illness began six weeks before admission, when shortness of breath upon exertion was first noticed. This became progressively more marked. Patient had some cough.

On admission there was present general œdema of lungs, abdomen, face and extremities with some cyanosis. Heart was enlarged but no organic lesion made out. Radial walls markedly thickened. Urine contained hyaline and granular casts and 0.2-0.7 per cent. albumin. Condition became gradually worse. Cheyne-Stokes breathing appeared and patient died seven weeks after admission, after illness of about three months.

Anatomical diagnosis. Arteriosclerosis; cardiac hypertrophy; thickening of mitral valve; extensive supra-arterial epicardial fibroid nodules; thrombi in auricular appendages; infarct of kidney; chronic nephritis; œdema and chronic passive congestion.

Additional notes. Heart weighed 540 grms. Hypertrophy, chiefly of left ventricle, with dilatation. Over the epicardial surface of the main coronary arteries of the left ventricle, as well as their branches and small twigs, are seated, discrete, small, elevated fibroid nodules of a semi-translucent or opaque appearance, resembling often beads strung along the course of the superficial vessels (Plate XI). Similar nodules are found in small numbers upon the auricles and upon the arch of the aorta. In these situations their relation to blood-vessels can also be demonstrated. Several similar nodules occur along the course of the larger branches of the coronary vein (Plate XI).

The coronary arteries are tortuous and extensively sclerosed. There is a general arteriosclerosis.

DESCRIPTION OF PLATES IX–XI.

PLATE IX.

Fig. 1 (Case II).—Transverse section of small artery in epicardium, surmounted by a fibroid nodule. The fibrous thickening is over about one-half the width of the vessel. Hæmatoxylin and eosin staining. *A.* Intima. *B.* Inner elastic coat; wavy lines showing only at the sharper turns of the vessel wall. *C.* Media, slight swelling and degeneration (reduction in the number of nuclei) beneath the nodule. *I.* Adventitial coat unaltered. *F.* Normal epicardium. *G.* Supra-arterial fibroid nodule projecting above the surface of the epicardium and extending to the adventitial membrane. *J.* Accumulation of small round cells in the epicardium at the sides of the node. *K.* Nerves in cross-section. *L.* Capillaries. *M.* Portion of a vein evidently not associated with the fibrous nodule. *H.* Heart muscle.

Fig. 2 (Case II).—Specimen stained in fuchsin (Manchot's method). *A.* Intima. *B.* Inner elastic coat unaltered; no break or weakness beneath the nodule. *C.* Media. *D.* Outer elastic membrane between the muscle and the adventitial layer. This elastic membrane is thick and firm, and evidently supplying the chief support and giving the elasticity to the vessel wall. The membrane is intact, except at *E*, where beneath the nodule there is a marked fraying out and disappearance of the elastic fibres with a consequent diminution in the strength of the arterial wall. *F*, *G*, *H*, *I* as in Fig. 1.

PLATE X.

Fig. 1 (Case II).—Same specimen as Plate IX, Fig. 2, stained by hæmatoxylin and eosin. *A.* Intima, with areas of proliferation probably not associated with the fibrous nodule. *B.* Inner elastic coat. *C.* Media apparently unchanged. *I.* Adventitial coat unaltered. *F.* Normal epicardium. *G.* Fibroid nodule projecting but slightly above the surface of the epicardium and extending to the adventitial coat beneath. *J.* Groups of small round cells at the side and beneath fibrous nodule. *K*, *L*, *M*, *H* as in Plate IX, Fig. 1.

Fig. 2 (Case III).—Transverse section of larger artery surmounted by a pronounced fibrous thickening, somewhat pedunculated and projecting abruptly from the surface. Same staining as Plate IX, Fig. 2. *A.* Intima. *B.* Inner elastic coat, very firm, unaltered. *C.* Media. *D.* Outer elastic coat, compact only for a small portion of the circumference on the myocardial side. From this point on each side the fibres may be seen to separate into a network and to become diminished in number until at *I* they cannot be demonstrated.

PLATE XI.

Heart of Case V.—The supra-arterial epicardial nodules are well shown over the coronary arteries and their branches. To the left are seen a few nodules upon a large vein.

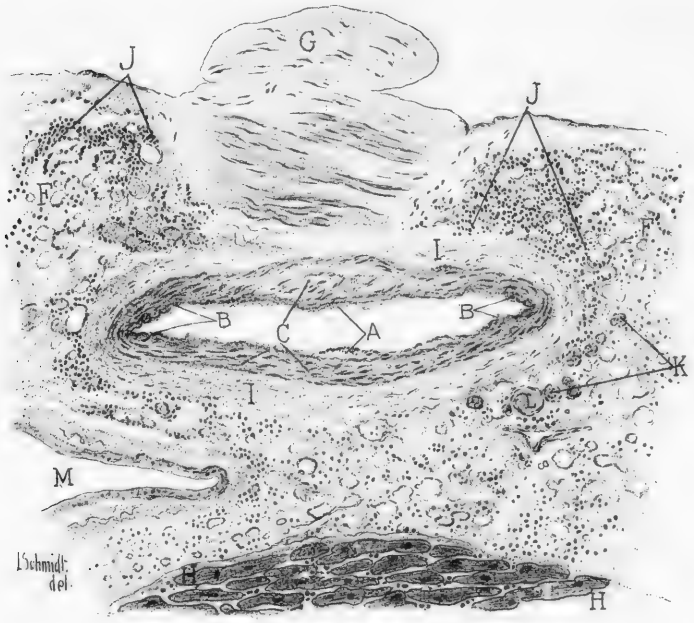

FIG. 1.

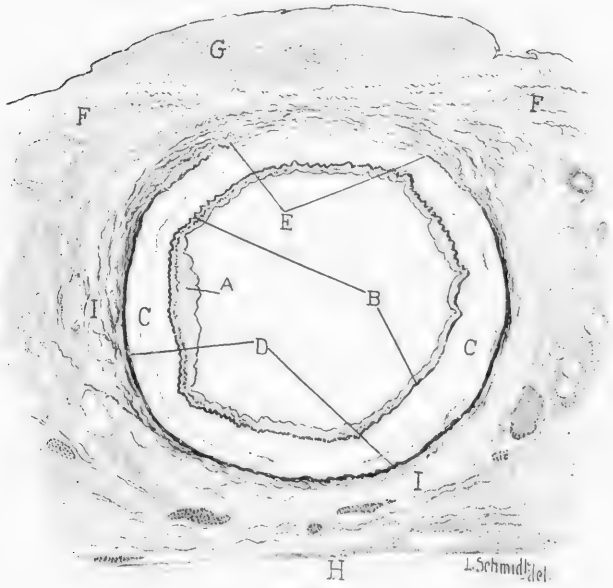

FIG. 2.

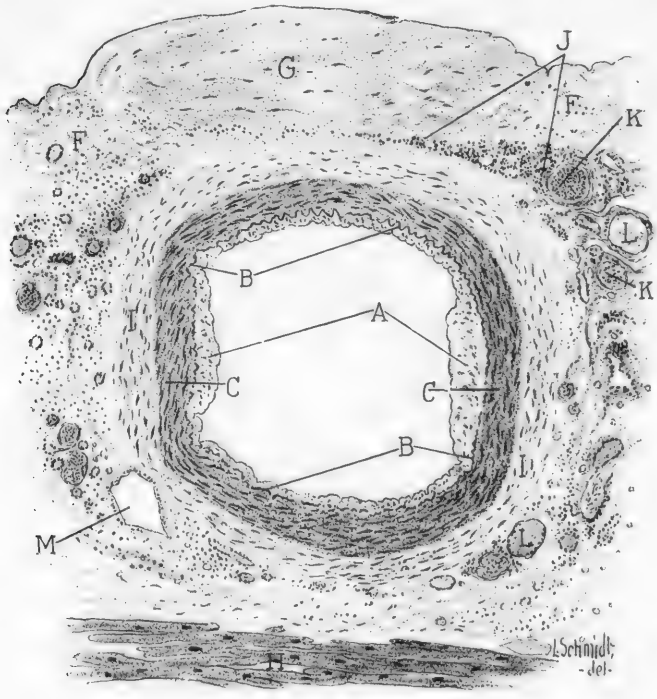
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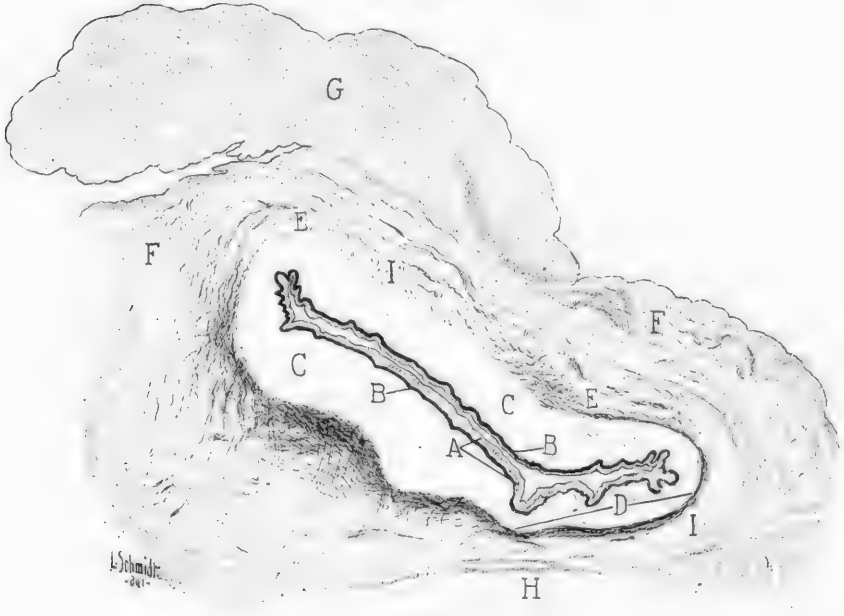
FIG. 1.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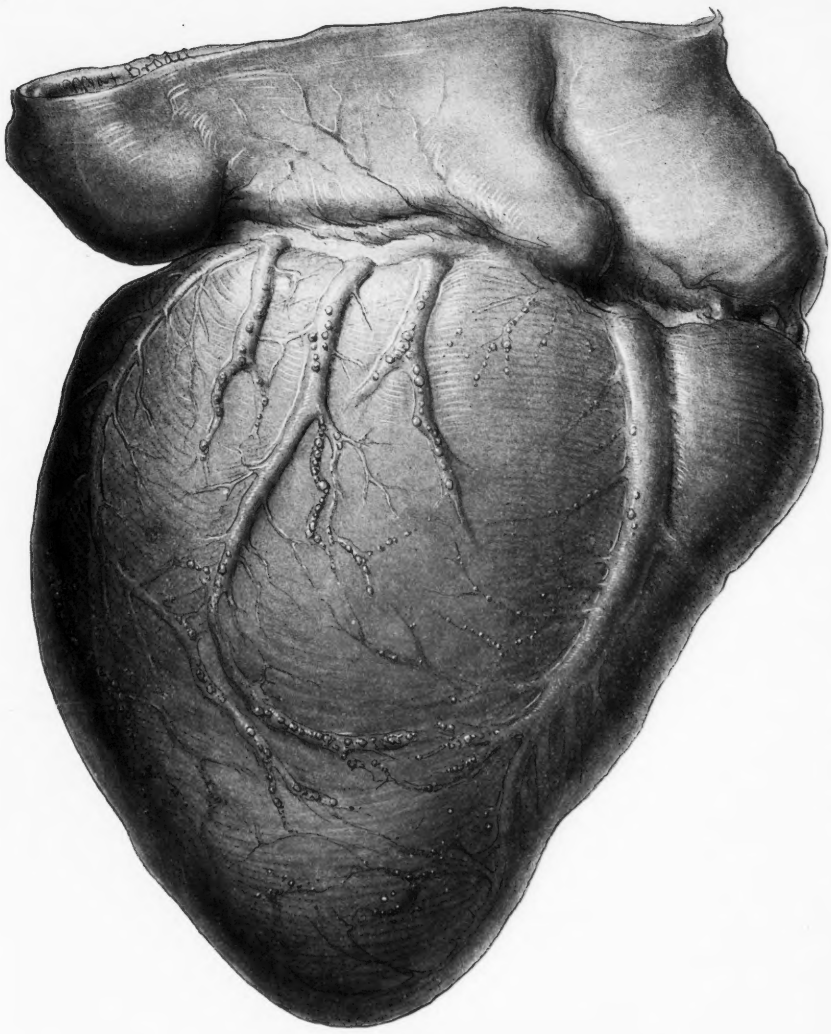
FIG. 2.